(PUBLISHED QUARTERLY)

Journal of the Council for Scientific and Industrial Research.

Vol. 6. NOVEMBER, 1933. No. 4.

Citricultural Research.

Investigations at the Commonwealth Research Station, Griffith.

By E. S. West, B.Sc. (Adel.), M.S. (Calif.)

1. Introduction.

The Commonwealth Research Station, Griffith, was established in 1924 on an area of 57 acres situated in a central position 3 miles from the town of Griffith, and made available by the Water Conservation and Irrigation Commission of New South Wales, which initiated the movement for the establishment of the Station, and which has, since its inception, contributed towards its upkeep. The Commission is kept in touch with the work through a liaison officer. In addition to the Commission, the local settlers at Griffith have also helped in the work in various ways, in the early days through a body they had set up and known as the Murrumbidgee Irrigation Areas Research Bureau, and lately through a local Advisory Committee.

The Station is surrounded by some 829 fruit-growers who have between them some 4,830 acres planted to citrus. In addition, many of the results of the Station will naturally be applied to other citrus-producing areas of the Commonwealth.

An account of the early work of the Station was given some years ago (see this *Journal,* 1: 95 and 353). Since that time, further progress has been made in various directions, the more important of which are indicated in the paragraphs that follow.

2. Present Organization of Station.

Equipment.—From time to time in the past few years, small additions have been made to the buildings and equipment of the Station. The most important of these has been a small chemical laboratory where the necessary analyses of soils, &c., can be carried out. Of late, the Station has also served as the headquarters of an officer of the Division of Soils who has been making soil surveys in the Areas.

The following buildings now exist on the Station:—A chemical laboratory, equipped for soil chemistry and consisting of five rooms, a store-room, and a dark-room; a small detached iron building, consisting of a soil storage and preparation room and a still room; three residences for members of the staff; an implement shed and workshop; stables; an old weatherboard building of four small rooms used as a store-house; and a large spray irrigation plant. The laboratory and cottages are fitted with electricity, water supply, and sewerage services.

Certain meteorological observations have been taken at the Station since its inception, but in 1931 it became the Commonwealth Meteorological Bureau's official recording station for the Griffith district.

Arrangement of Planted Area.—The area of the Station amounts to nearly 60 acres (of which 50 are irrigable). The area is divided up into fields as per the plan in Fig. 1. Of the area available, about 30 acres have been planted to citrus, 10 acres to lucerne, and another field of 10 acres has been partly utilized for experiments in tick bean seed production.

Staff.—The present staff of the Station is as follows:—

Officer-in-Charge ..	Mr. E. S. West, B.Sc. (Adel.) M.S. (Calif.)
Field Research Officer ..	Mr. R. R. Pennefather, B.Agr.Sc. (Melb.)
Chemist	Mr. A. Howard, M.Sc. (Melb.)
Orchard Superintendent..	Mr. B. H. Martin, Dip. Hawk. Coll.
Farm Foreman ..	Mr. T. J. Masters.
Clerical Assistant ..	Miss E. Beck.
Liaison Officer (C.S.I.R. and W.C.I.C.)	Mr. F. K. Watson, M.A., B.Sc.Agr., B.Sc. (Eng.), A.M.I.C.E. (London), A.M.I.E. (Aust.).

3. Present Investigations.

In the spring of 1924, the three original fields were planted, namely, (i) a soil treatment field, (ii) a green manure experiment field, and (iii) a fertilizer field. These were designed to answer the cultural questions that appeared most urgent at the time, but the work of the Station has since developed in other directions as well. All the trees in these fields were raised from selected buds of Washington Navel parent trees and Late Valencia parent trees. Experience has shown that the choice of buds was very satisfactory, as fruit from the different trees is of a uniformly good quality and size.

Soil Treatment Field.—At the time of the establishment of the Station, great difficulty existed in the management of the heavy retentive soils which made up a considerable portion of the Murrumbidgee Irrigation Areas. During wet winters, and particularly that of 1923, trees died through water-logging of the soil, cultivation of the soil seemed to be difficult, and it was considered that the impervious nature of the clay band which underlay the surface soil prevented the proper penetration of the roots of orchard trees and also the penetration of irrigation water. Successful management of such soils, therefore,

seemed to be the most pressing necessity of the moment, so that plots were laid out in this field to test the possibility of overcoming these difficulties by the use of tile drains, deep ploughing, subsoiling, and by applications of lime and of gypsum.

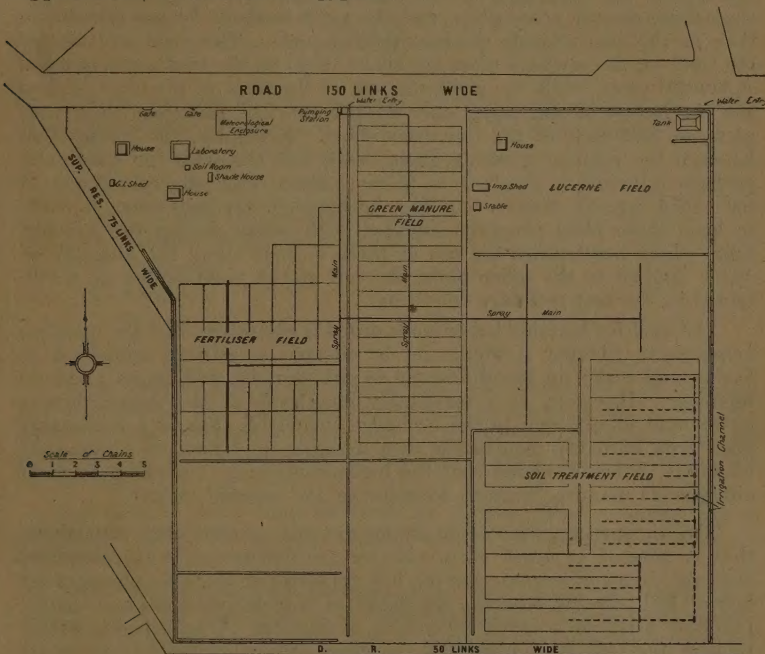

Fig. 1.—Plan of the Commonwealth Research Station, Griffith, N.S.W.

The work of the Station and of the Irrigation Commission goes to show that these troubles can be controlled by simple and less expensive methods. Thus a high water table can be easily lowered by growing deep-rooted green crops between the citrus trees. Then again it has been found that by improved methods of irrigation, water-logging can be prevented, so that the problems associated with these heavy retentive soils have largely been overcome.

Green Manure Field.—Five green manure treatments were originally selected, namely :—

(1) Summer green manure crop (cowpeas).
(2) Winter green manure crop (tick beans).
(3) Biennial green manure crop (Bokhara clover).
(4) Perennial green crop (lucerne) .
(5) Continuous clean culture as a control.

Results have now been obtained over a sufficient number of years on which to base reliable conclusions. Very early during the growth of the trees, it was noted that those on the winter green manure plots were making better headway than the others. Later, it was noted that

trees in the summer growing crop plots, viz., cowpeas and lucerne, were growing more slowly than those on the clean cultivated plots. Using the diameter of the butts of the trees as an index to size, it has been found that the trees on the winter green manure crop plots are larger than those on any other plots, and the yield is about 30 per cent. more than on the continuously clean cultivated plots. The yield and size of the trees on the cowpeas plots are below those on the continuously clean cultivated plots, while the yield and size of the trees on the lucerne plots are the lowest of all, being only 66 per cent. of the clean cultivated plots, or half that of the tick bean plots. Evidently, the competition between the summer growing green crops and the trees for water and perhaps minerals is so great as to more than offset the advantages of the added organic matter. In fact, although every endeavour is made to keep these plots properly supplied with water during the summer time, which necessitates the use of three or four times the quantity of water applied to the other plots, the soil of the plots cannot be maintained in the best moisture condition.

The seed for suitable leguminous crops is expensive, and the question arises as to whether it would not be cheaper to grow a crop such as barley and make up the difference in nitrogen by adding an artificial fertilizer. However, as a reasonably good crop of tick beans returns an amount of nitrogen to the soil over and above that of a reasonably good crop of barley equivalent to a dressing of 7 cwt. of sulphate of ammonia, it is considered that the leguminous crop is a cheap form of nitrogenous manure, without considering the organic matter.

The nitrogen question is an important one, particularly with citrus that is affected so much when nitrates are deficient. It is recognized that the decay of organic matter low in nitrogen leads to a temporary loss of soil nitrates, whereas the decay of nitrogen-rich organic matter probably leads to a quick formation of nitrates. The nitrate content in both the tick bean plots and the clean cultivated plots is therefore being followed week by week. The investigation has just begun, but promises to yield interesting information. It is hoped to extend the work to include the cowpeas plots later, as on these plots the legume is growing and the green stuff decaying at different seasons of the year from those of the tick beans.

After several years of persistent growth and turning under of green manures, it is now observed that the structure of the soil has been definitely changed. A different type of cracking occurs on the green manure crops from the clean cultivated crops, but what is of more practical importance, it is being found to be increasingly difficult to get sufficient water to soak into the clean cultivated plots at irrigations, whereas the green manure plots still absorb water quite readily. It is a very common observation that, after the soil has been cultivated and irrigated for several years, the structure deteriorates, e.g., soil that once appeared loose and open becomes stiff and compact. Evidently, the annual growth and turning under of green manure is a remedy or preventative, and for this reason alone the use of green manure is desirable.

Since 1928, when the value of tick beans as a winter green manure was first established by this experiment, the use of such beans throughout the Areas has doubled itself in every succeeding year, so that now it is rapidly becoming a standard practice of the district.

The Station is at present giving some attention to other winter legumes, for example, Berseem clover, lupins, tares and tick beans, tares and barley, field peas and tick beans, and field peas and barley, to ascertain whether tick beans alone can be improved on. Trials are also being made in regard to the question of growing tick beans locally for seed in order that the existing cost of seed to growers may be reduced.

Mineral Fertilizers.—The original mineral fertilizer field was established to ascertain the relative values of the following treatments:—

1. Nitrogen, phosphoric acid, and potash.
2. Nitrogen and potash.
3. Nitrogen and phosphoric acid.
4. Phosphoric acid and potash.
5. Nitrogen.
6. Potash.
7. Phosphoric acid.
8. No fertilizer.

Up to date, no significant differences in the size or yield of the trees are noticeable. This is rather surprising, in view of the very early benefit found from green manuring. Evidently, the organic matter added to the soil is important to the plant, apart altogether from the nitrogen.

It has been noted that the trees on all the plots receiving superphosphate develop a mottling, regardless of whether or not nitrogen or potash is added as well.

Experiments carried out on older groves have shown that citrus trees respond to heavy dressings of nitrogenous fertilizers, and these experiments have led to the extensive use of sulphate of ammonia on mature trees throughout the Areas.

Bud Selection.—Judging from the results that have been obtained with the selected trees of the Station, there is reason to believe that much of the trouble often met with in young Navel oranges is due to inferior types. Many of these types are characterized by large coarse-skinned fruit, which characteristic is greatly intensified when the tree is young or is making great vegetative growth. The true Washington Navel type also shows this tendency but to a much less extent, so that the fruit is not very coarse even when the tree is young.

Phosphatic Fertilizer Experiment with Lucerne.—In the autumn of the year 1926, an experiment concerning the phosphatic manuring of lucerne was commenced. Plots receive annual dressings of 1 cwt., 2 cwt., and 4 cwt. of superphosphate per annum. There are also plots in which 1 ton of rock phosphate per acre was applied as an initial dressing only. The yields, while increasing with the amount of superphosphate, have also shown that dressings heavier than 4 cwt. would still be profitable. Unfertilized lucerne proved almost a failure, and the stand practically died out in two years. After six years, the stand of lucerne in the 1 cwt. plots still exists, but it cannot be said to be a good one. The stands in the 2 cwt. and 4 cwt. plots are good, but the latter yields about 7 tons of hay compared with 4 tons from the 2 cwt.

The behaviour of the rock phosphate plot has been interesting. The first year the yield was equal to that of the 1 cwt. superphosphate dressing, the second year it was better than the 1 cwt. dressing, and since then has been practically equal to the 2 cwt. superphosphate dressing.

Ripening of Oranges.—By determining the acid content of the juice and noting the colour of the rind of the oranges from the differently treated plots, some idea of the effect of different treatments on the ripening of the fruit is being obtained.

During the 1932 season, it was found that, in the green manure field, trees on the tick bean plots and clean cultivated plots coloured earlier and contained less acid than the trees on the Bokhara clover plots and lucerne plots; that is the larger and more vigorous the trees the earlier they coloured and lost their acidity.

Results obtained during the present season have confirmed this; in fact, the differences have been more striking, but as the season has a great effect on ripening, it is possible that it may be found that these effects are either intensified or otherwise in subsequent seasons.

Soil Moisture Investigations.—Whilst crop control of excess water and salt can prevent much damage, especially in certain badly situated areas, it early became evident that most of the trouble could be prevented at its chief source, viz., the actual application of irrigation water. In the early days of the Areas, scarcely any data were available for guidance, and excessive waterings were generally the rule. Accordingly, the Station has given considerable attention to the fundamental problems of the relationships between soil and moisture, and to such soil constants as the "wilting coefficient," "sticky point," and "field capacity." This work is facilitated by test wells (2 in. auger holes 11 ft. deep protected with casings of perforated down pipe) that are distributed throughout the various fields, and by means of a self-recording test well installed in a column of soil 1 metre in diameter and 6 metres deep isolated *in situ* and enclosed in an impervious jacket. The water table in the soil column is controlled by adding rain water to the surface of the soil in amounts and at times that circumstances indicate.

It has been found that the water table in soils is very sensitive to changes in atmospheric pressure and in soil temperature. Thus a lowering in the atmospheric pressure causes a rise of the water table as does an increase in the soil temperature. It is due to the first-mentioned of these effects that the increased flow of springs and seepages during the passage of cyclones is due.

In field studies involving the evaluation of the moisture content of the soil, a great difficulty is the large sampling error involved owing to the big variation in the physical texture in soils, which may, nevertheless, appear quite uniform. The use of the "sticky point" has been developed to correct for the error due to the variation in soil texture. The sticky-point is that moisture content of the soil at which the plastic mass just fails to adhere to external objects, and can be easily determined with sufficient accuracy. It has been found that with Griffith soils, the sticky point closely approximates the "field capacity," or that quantity of water that remains in the soil in the field after water has been added and approximate equilibrium conditions have been

attained. By subtracting the actual moisture content from the sticky point, we find the amount of moisture that must be added to the soil to again bring it to the field capacity, regardless of the texture of the soil. By this procedure, the precision of field moisture determinations is greatly increased.

The work at Griffith relating to the soil and its moisture content, results of which have been published as the Council's Bulletin 74, together with the intensive work carried out by the Irrigation Commission on the question of seepage and waterlogging, has had a very great influence on the irrigation practices of the Murrumbidgee Irrigation Areas. It is now generally recognized that care is necessary in handling water if the soils are not to be damaged, and very great advances are noticeable in the general irrigation methods and outlook of the local settlers.

Irrigation Methods.—The methods of applying irrigation water to the soil by means of flooding in various ways and by overhead sprays have been investigated for some time.

Amongst the surface methods, that depending on the application of water by allowing it to flow down furrows is perhaps the cheapest and easiest, but, in some types of soils, difficulties arise owing to excessive soaking at the top end of the furrow and to unequal wetting of the soil in the furrows and between the furrows. By increasing the number of furrows till they form a series of ridges and hollows from tree to tree, by suitably limiting their length, and by other modifications, these objections can be largely overcome. This work, however, necessitates the development of implements than can draw furrows under the foliage of trees. In co-operation with manufacturing firms, an endeavour is being made to design equipment for this purpose.

A second method of surface irrigation, namely, the border method, in which water flows down between low levees or borders, gives the required control over water, but it is difficult where the land slopes across the line of watering as well as down the line. For this reason it is not always applicable.

In the basin method, whereby the land is divided by levees into small basins, absolute control over the water is possible. Here again, however, rather level land is necessary, and the labour and cost of forming the levees may be too high for local conditions. Nevertheless, the method is being investigated.

The spray method of irrigation gives absolute control of the water applied under all circumstances, but the cost of its initial installation is rather high. Work aimed at the improvement of the existing apparatus, and the reduction of its initial cost, is in progress at the Station. The equipment under test was designed by a local grower, and is known as the "Kook" equipment after his name.

The previous initial cost of the equipment has already been reduced, and has been brought within the range of commercial practice. As a matter of fact, at the present time eleven large scale growers have installed overhead spray equipment on their blocks.

During the present (1933) winter, it is intended to investigate the effects of varying the lengths of laterals, spacings of perforations, size

of perforations, and pressure of water, so that graphs may be drawn to obtain information enabling the most economical equipment for any particular farm to be designed.

Other Investigations.—In addition to the foregoing work, the Station has a few other investigations in hand.

The effect of a soil mulch on moisture conservation and on soil temperature has been studied, and the results of this work have been published (see this *Journal* **3**: 97, 1930, and **5**: 236, 1932).

The Station's work on frosts carried out in association with the Irrigation Commission has demonstrated that the close proximity of mallee scrub is conducive to frost formation; the results have also been published (this *Journal* **4**: 173, 1931, and **6**: 80, 1933). Methods of protecting young citrus trees from frosts have been investigated, and it has been found that wrapping the stems with newspapers and mounding up with soil is a useful protection. It is also of great importance to maintain a vigorous growth during the summer.

On the Murrumbidgee Irrigation Areas, the Valencia orange possesses the objectionable habit, well known in many varieties of fruit, of bearing alternate heavy and light crops. This not only causes serious marketing difficulties, but the quality of the fruit also suffers. Undoubtedly the same general principles are at work here as in other fruit, such as the apple, but the details differ, as the period between fruit bud formation and setting in the orange is the matter of a few weeks only. It seems significant that the fruit of one seasons' crop of Valencias is still on the tree when the next season's crop is setting. The question is being investigated in co-operation with the Division of Plant Industry.

Finally, the post-war expansion of the Australian citrus industry has entailed a greater amount of attention being given to marketing problems, particularly those of the storage and transport of the fruit. The great bulk of Australia's citrus exports are derived from the Griffith district. The Station is, therefore, in a particularly favorable position to study many important features of the problems in question, particularly from the point of view of the effects of conditions in the groves. Cool storage tests with Navel oranges are now being carried out by the Station on behalf of the Citrus Preservation Committee. The necessary storage facilities have been made available by the Griffith Producers' Co-operative Company Limited.

The Use of Carbon Dioxide in the Storage of Chilled Beef.

By W. A. Empey, B.V.Sc., and J. R. Vickery, M.Sc., Ph.D.†*

The work described in the article that follows forms part of the programme which is being carried out by the Council's Section of Food Preservation and Transport in co-operation with the Queensland Meat Industry Board. The lines of that co-operation and some details regarding the programme itself, have been given in a previous issue (this *Journal* 5: 133, 1932). Briefly, the Meat Industry Board has provided the buildings and equipment for the Section's laboratory at the Brisbane Abattoir, Cannon Hill, Brisbane, while the Council is supplying and maintaining the necessary research workers.—Ed.

Summary.

Under the conditions in which the experiments were carried out, attack on the moister superficial tissues by bacteria—mainly various strains of *Achromobacter*—has been the chief cause of wastage in chilled beef stored for prolonged periods. Deterioration caused by the growth of moulds has been relatively insignificant, due mainly to the fact that moulds have constituted an extremely small percentage of the initial contamination.

2. The duration of safe storage, both in carbon dioxide and in air, was largely determined by the numbers of bacteria acquired by the beef during dressing and initial chilling and capable of relatively rapid proliferation at a temperature of —1°C. (30.2°F.).

3. The extent to which small concentrations of carbon dioxide, of the order of 10 to 12 per cent., in the storage environment restricted the rate of growth of *Achromobacter* determined the length of storage possible without appreciable deterioration occurring in the beef, and this period was approximately 40 per cent. greater than that possible under similar conditions of storage in air.

4. Beef obtained from meat works in which *Achromobacter* constitutes the main type of initial infection, may safely be held in the chilled condition for a period of 53 days (approximately equivalent to a period of transport of 45 days), provided that strict hygenic conditions are maintained during slaughter, dressing, and chilling, in order to ensure an extremely low bacterial infection, and provided also that concentrations of carbon dioxide, of the order of 12 per cent., be employed in the storage environment.

1. Introduction.

For countries regularly engaged in a chilled beef trade with Great Britain, the duration of the voyage seldom exceeds 25 days, but the corresponding time from Queensland, which contributes rather more than 85 per cent. of Australia's exports of quarters of beef, at present exceeds 50 days. Since about five days for the preparation of the beef in the meat works, and about three days for its marketing, must be added to this period, the average duration of holding of chilled beef exported from Queensland is likely to be of the order of 60 days. Only a small margin of safety exists in the export of chilled beef from countries nearer to Great Britain. It therefore seems fairly certain that, in

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order to secure reasonable freedom from wastage, a somewhat different technique from that used by Australia's competitors must be employed in the export of chilled beef from the northern States of Australia.

Investigations carried out in Great Britain, Germany, and the United States of America, and confirmed in this laboratory, have shown that deterioration of beef during storage at temperatures close to the freezing point of the meat, —1°C. (30.2° F.), is due almost wholly to the proliferation of certain species of bacteria and moulds on the superficial tissues of the quarters. The extent of deterioration during storage therefore depends both on the degree of contamination of the beef by low-temperature type bacteria and moulds* during slaughter, dressing, handling, chilling, and storage, and on their rates of proliferation during chilling and storage. As investigations on the first factor, discussed in detail in subsequent sections of this communication, have shown that the adoption of simple precautions will reduce, but not wholly eliminate, the initial microbial contamination, adequate control of the rates of proliferation of the "low temperature type" species during chilling and storage is therefore essential.

Brown(2) showed that relatively high concentrations of carbon dioxide in the air of the storage environment gave complete inhibition of the growth of many species of moulds, and Killiffer(7) found that the duration of storage of many types of fresh meat products could be greatly prolonged by the use of relatively high concentrations of carbon dioxide. The maintenance of high concentrations of this gas—say 50 per cent.—in ship's holds and other stores presents almost insuperable difficulties, and, therefore, detailed studies of the effects of relatively low concentrations on the growth of "low temperature type" moulds and bacteria have been carried out by many investigators working in the laboratories of the British Food Investigation Board (3, 4, 5, 9, 10). These studies have shown that concentrations of carbon dioxide, of the order of 10 to 20 per cent., produce an inhibitory effect on the growth of most micro-organisms commonly found on meat and fish.

As it was impossible to anticipate the problems involved in projecting the results of laboratory and small-scale experiments to a commercial scale, experiments, wherein quarters of beef were stored in atmospheres containing 10 to 12 per cent. carbon dioxide, have been carried out under strictly controlled commercial conditions, in conjunction with a large meat exporting works. The use of carbon dioxide during initial chilling of beef to the temperature of storage being impracticable, its effects during storage only have been studied.

2. Experimental Procedure.

(i) *General.*—Six bodies of beef of average quality were employed in each experiment, and the customary abattoir procedure for their dressing, handling, and chilling was followed. In the first experiment, however, the beef was chilled in a large commercial room, and, in the second, in a small experimental chamber. After the completion of chilling to a uniform temperature of 0°C. (32°F.), the sides were divided into quarters which were covered with sterilized stockinette, and placed in two storage chambers cooled by overhead grids of brine pipes and

* Bacteria and moulds capable of comparatively rapid growth under suitable conditions at temperatures of the order of —1° C. (30.2° F.) will subsequently be referred to as "low-temperature types."

maintained at similar temperatures and relative humidities. The construction of these chambers was similar in all respects, except that one was made "gas-tight" to enable uniform concentrations of carbon dioxide to be maintained in its atmosphere. Comparable experimental material was obtained by placing the twelve left quarters in the "gas" chamber, and the twelve opposite quarters in the control chamber.

Only the main features of the experimental procedure are described.

(ii) *Physical Conditions.*—Losses of weight during chilling and storage were obtained. In Test 2, the extent of superficial desiccation of exposed muscle tissue was determined by measuring the moisture contents of successive 2 mm. layers taken immediately after chilling and at the completion of storage from four exposed areas of muscle in the vicinity of the aitch bone of the hindquarters; the observations had to be limited to this region owing to its comprising the bulk of the small area of exposed muscle available on each side of beef. From time to time the moisture contents of the first half millimetre (outer) layer of exposed muscle were determined.

During the progress of chilling, continuous observations were made of the average temperature, relative humidity, and rate of movement of the air, and of the rate of reduction of the temperature of the deep and superficial portions of the meat.

Observations of the temperature, relative humidity, and concentration of carbon dioxide in the atmosphere of each chamber, and of the temperature of the superficial portions of the meat, were carried out twice daily, and were so determined as to obviate the necessity of opening the chambers, except at such times as detailed examination of the meat was desirable. The temperatures of the air and of the meat in each chamber were determined at six and three positions respectively. While cold brine was circulating through the grids of pipes, the concentrations of carbon dioxide at different positions in the chamber did not vary from the mean value by more than ± 0.1 per cent.; the concentration at one point only was therefore determined.

(iii) *Sampling for Microbial Examinations.*—Since deterioration affected only the superficial tissues, samples of exposed muscle and adipose tissue, each 2 sq. cm. in area, were excised to a depth of 2 mm. for the determination of the type and numbers of the microbial population. The initial sampling was carried out within one or two hours of slaughter, six areas each of exposed muscle and adipose tissue being removed, under aseptic conditions, from each of four sides of beef; the 24 samples of muscle and of adipose tissue were combined in order to give an average value of the microbial contamination of each type of superficial tissue. Similar samples from contiguous areas were removed, in one experiment, at the completion of chilling, and, in both experiments, on several occasions during storage. The percentage of "low temperature type" micro-organisms present in each group of samples was determined by comparing the numbers of organisms viable on artificial media at incubation temperatures of 20° C. (68° F) and —1° C. (30.2°F).

(iv) *Sampling for Examination of Fat.*—At the completion of chilling and of storage, three samples of superficial fat, each 2 mm. thick

and 40 sq. cm. (approx.) in area, were removed from the back, abdominal, and neck-thorax regions of six quarters. The 18 samples of each group were subsequently minced and ground in a mortar to secure intimate mixing. At the completion of storage, samples were also obtained from areas heavily infected by either bacteria or moulds. Estimations of the acid values of the extracted fat and degree of rancidity of the "native" fat, determined by the measurement of the "active" oxygen value described by Lea (8), were carried out on each of the mixed samples. "Blind" palatability tests, by three observers, on hot and cold portions of each of the mixed samples of fat served to detect the presence of "off" flavours. For these tests, tissue sampled immediately after the completion of chilling was stored in stoppered bottles at a temperature of —17° C. (1.4° F.) pending the comparison with the samples obtained at the completion of storage.

3. General Observations on Storage Experiments.

(i) *Physical Data.*—Table I. gives a summary of the physical conditions obtaining during the chilling of the beef (in sides). The duration of holding in the chilling rooms was 72 hours (approx.).

TABLE I.—SUMMARY OF THE PHYSICAL CONDITIONS DURING STORAGE.

Test No.	Mean Air Temp. ° C.		Mean Relative Humidity Centre Room.	Mean Vr (mm. Hg.).		Mean (Vm-Vr) (mm. Hg.).		Reduction of Meat Temperatures. Av. Time Taken. (Hrs.)		Air Movement. Time (mins.) for One Complete Change.	Weight Loss.	Original Moisture Lost. (Mixed S.M.)
	First 8 Hours.	Subsequent Cooling Period.		First 8 Hours.	Subsequent 24 Hours.	First 8 Hours.	Subsequent 24 Hours.	Deep Butt to Reach 5° C.	Mean superficial to Reach 10° C.			
			(%)								(%)	(%)
1	2·3	—0·5	94	6·0	4·5	16·6	1·9	47	13	2·5	0·6	17
2	5·9	0·0	78	5·3	3·7	15·5	2·5	41	8	0·75	1·2	27

Vr = Aqueous vapour pressure at centre of room.
Vm = Saturation vapour pressure at mean superficial temperature of the beef.
S.M. = First ½ mm. layer of exposed muscle. (Average moisture content fresh muscle is 75 per cent.)

The rate of cooling of the beef, particularly in the superficial areas, was considerably greater in Test 2 than in Test 1. In Test 2, the higher rate of movement and lower vapour pressure of the air is reflected both in the greater total loss of weight and in the lower average moisture content of the first half-millimetre (outer) layer of the exposed muscle.

The chief physical data observed during the storage of the beef are collected in Table II.

Table II.—Physical Data during Storage.

Test No.	Duration of Storage (days).	Air Temp. °C. Mean.	Air Temp. °C. Mean Deviation.	Air Temp. °C. Mean Spatial Difference.	Superficial Meat. Temp. °C. Mean.	Superficial Meat. Temp. °C. Mean Deviation.	Superficial Meat. Temp. °C. Maximum Deviation.	Mean Relative Humidity.	Vr (mm. Hg.).	Vm-Vr (mm. Hg.).	CO_2 Concentration. (%) Mean.	CO_2 Concentration. (%) Mean Deviation.	Weight Loss Per 44 Days During Storage.
								%					%
1. "Gas" store	44	$-1{\cdot}0_0$	$0{\cdot}1_5$	$\pm 0{\cdot}3_0$	$-0{\cdot}9_0$	$0{\cdot}1_0$	$+0{\cdot}1_5$ $-0{\cdot}2_0$	96	$4{\cdot}1_5$	$0{\cdot}1_5$	$10{\cdot}2_5$	$0{\cdot}2_5$	2·2
Control Store	29	$-1{\cdot}0_0$	$0{\cdot}1_0$	$\pm 0{\cdot}3_0$	$-0{\cdot}8_5$	$0{\cdot}0_5$	$\pm 0{\cdot}2_0$	96	$4{\cdot}1_0$	$0{\cdot}2_0$	$0{\cdot}0_5$	..	..
2. "Gas" Store	55	$-0{\cdot}9_0$	$0{\cdot}1_5$	$\pm 0{\cdot}3_0$	$-1{\cdot}0_0$	$0{\cdot}0_5$	$+0{\cdot}2_0$ $-0{\cdot}1_5$	93	$4{\cdot}0_0$	$0{\cdot}2_5$	$12{\cdot}1_0$	$0{\cdot}4_0$	2·7
Control Store	55 (x)	$-0{\cdot}9_5$	$0{\cdot}1_5$	$\pm 0{\cdot}3_0$	$-0{\cdot}9_0$	$0{\cdot}1_0$	$\pm 0{\cdot}2_0$	94	$4{\cdot}0_5$	$0{\cdot}2_5$	$0{\cdot}1_0$	..	2·7

(x) Eight of twelve quarters were removed after storage for 40 days.

Vr = Mean aqueous vapour pressure.

Vm = Saturation vapour pressure at the temperature of the surface of the meat.

As it was desired to prevent any undue formation of ice in the muscle tissue of the beef during storage, the mean air temperature could not be maintained lower than —1.0°C. (30.2°F.)—the average freezing point of beef muscle—particularly in view of the fact that the coldest part of the storage rooms had a steady temperature of —1.3°C. (29.7°F.). In such positions, after a period of storage in excess of 40 days, the quarters of beef invariably showed some formation of ice in the muscle tissue. The slight divergence of the mean meat temperatures from the mean air temperatures was due to there being an insufficient number of thermometers in the superficial tissues of the meat to permit the estimation of their true average temperatures.

In both experiments, no forced air circulation was employed during storage, other than that required for a period of ten minutes daily, while carbon dioxide was being added to maintain the requisite concentration. For purposes of comparison, the losses of weight of the quarters during storage, assumed to vary directly with time, have been reduced to a common basis of holding for 44 days; tests have shown this assumption to be reasonably accurate.

In Test 2, the average moisture contents of successive 2 mm. layers of exposed muscle after chilling were respectively, 55, 63, 69 per cent.; after storage, the corresponding values were 60, 64, and 67 per cent. respectively. During storage, the superficial exposed muscle had apparently acquired fresh moisture by diffusion from the deeper layers, and the superficial muscle was moister at the completion of storage than it was at the completion of chilling. The presence of bacterial slime on the exposed muscle of the beef after storage prevented this procedure being adopted in the first test.

(ii) *Microbial Contamination.*—Table III. gives a summary of the chief features of the average microbial contamination (excluding yeasts), per sq. cm. of exposed tissue, of the quarters of beef employed in the two tests.

TABLE III.—MICROBIAL COUNTS EXPRESSED AS NUMBERS OF MICRO-ORGANISMS PER SQUARE CENTIMETRE OF EXPOSED TISSUE.

Test No.	Tissue.	Time (days) Since Slaughter.	Count of "Low Temperature Type" Organisms.			
			Air Stored.		CO_2 Stored.	
			Bacteria.	Moulds.	Bacteria.	Moulds.
1.	Muscle	0	550	<5	550	<5
		3	103,000	<50	103,000	<50
		31	$18{,}000 \times 10^6$	<1,000	600×10^6	<100
		46	..	..	$8{,}000 \times 10^6$	<500
	Fat ..	0	250	<5	250	<5
		3	32,000	<40	32,000	<40
		31	100×10^6	<200	70×10^6	<50
		46	..	..	500×10^6	<100
2.	Muscle	0·3	56	<1	56	<1
		21	80,000	16,000	400	<10
		42	1.5×10^6	0.1×10^6	0.02×10^6	<100
		57	150×10^6	1×10^6	0.4×10^6	<1,000
		57 + 2 at 10° C.	..	..	30×10^6	..
	Fat ..	0·3	30	<1	30	<1
		28	2,000	200	200	<10
		42	100,000	10,000	6,000	<50
		57	1×10^6	0.1×10^6	0.02×10^6	<500
		57 + 2 at 10° C.	..	..	12×10^6	..

In each test, visible microbial contamination first appeared as small, moist, bacterial colonies in the form of nodules, situated usually in the moister areas of exposed muscle on the neck, the residual tissues of the abdominal aorta, and beneath the diaphragm. During chilling and storage, the neck and aortal areas receive some drainage of blood and lymph from the adjacent vessels.

As the duration of storage increased, these colonies tended gradually to coalesce and to form "slime," which was invariably accompanied by a sour, stale odour. This point of definite onset of deterioration invariably occurred when the number of "low temperature type" bacteria per sq. cm. of exposed muscle was of the order of 5×10^7, an observation which agrees reasonably well with that of Haines(6), who specifies that the "slime" stage is reached in moist muscle when the number of organisms per sq. cm. is approximately 3.2×10^7. The times from slaughter required for such development in Test 1 were 23 and 16 days for the beef stored in carbon dioxide and air respectively, while the corresponding times in Test 2 were 67 (estimated) and 45 days

respectively. In Test 2, the "slime" stage was not reached at the conclusion of storage in 12 per cent. CO_2—57 days from slaughter—and the value given above has been estimated from bacterial counts. In Test 1, the onset of deterioration subsequent to the "slime" stage was marked by more extensive spreading and an increase in the size of the colonies in the areas of muscle where visible growth had first become apparent, and it was marked also by the appearance of similar slime on the relatively drier areas of muscle, on the connective tissue covering the abdominal and thoracic cavities, on the fat covered by moist connective tissues, and on the moist surfaces of the rib muscles exposed as the result of the division of sides into quarters.

An analysis of the species of "low temperature type" bacteria found initially on the surfaces of the beef showed that several types belonging to the genus *Achromobacter* Bergey, *et al.*, constituted at least 95 per cent. of the infection, and that the remainder consisted of several species of *Pseudomonas* and *Micrococcus*. During the subsequent development of nodules and slime, the relative percentages of *Achromobacter* and *Pseudomonas*, both on the beef stored in carbon dioxide and on its control stored in air, tended to increase at the expense of the slower growing *Micrococcus*.

An examination of the data given in Table III. shows that the extent of the restriction of bacterial proliferation, caused by the use of 10 to 12 per cent. carbon dioxide, varies considerably at different stages in the period of storage. From the point of view of the safe storage of chilled beef, however, the extent of inhibition up to the time of initial slime formation is obviously of paramount importance, and, during this period, the carbon dioxide has effected a reduction of 40 per cent. (approx.) in the rate of proliferation. Comparisons of the growth rates of *Achromobacter* (several strains) and *Pseudomonas* on plates of nutrient agar in atmospheres of 94 and 99 per cent. relative humidities, determined according to the method described by Coyne(4), showed that 10 to 12 per cent. carbon dioxide caused an inhibition of 30 to 40 per cent. at the stage when growth became abundant, and thereby confirmed the observations made directly on the beef.

In Test 2, the effect of holding beef for two days in ordinary atmospheric conditions (mean temperature 10°C. (50°F.)) subsequent to its storage in carbon dioxide was investigated. Although the bacterial count on the exposed muscle increased almost sixty-fold during this short period (see Table III.), the "slime" point was scarcely reached, even in the moister areas.

In all tests carried out in this laboratory—including many not described in this paper—the counts of "low temperature type" moulds per sq. cm. of exposed muscle and fat, obtained immediately after slaughter and after the completion of chilling, have been extremely low in comparison with the counts of "low temperature type" bacteria, and this condition has been reflected in the comparative absence of appreciable fungal attack, even after prolonged periods of storage. In the areas showing marked bacterial proliferation, fungal growth has invariably been absent.

In Test 2, after a period of storage of 42 days had elapsed, no visible colonies of mould were present on the beef stored in carbon dioxide, whereas the beef stored in air had an average population of 50 to 60

colonies, chiefly composed of *Penicillium expansum* with some *Sporotrichum carnis.* In the case of beef stored even so long as 55 days in 12 per cent. carbon dioxide, the number of visible colonies did not exceed three or four per quarter, and all proved to be *Sporotrichum carnis.* Since heavy bacterial contamination in all cases rendered necessary the disposal of the air-stored beef before mould colonies could be detected on the beef stored in carbon dioxide, it was impossible to make accurate estimates of the comparative rates of growth of the moulds on the meat in the two environments. Within the periods of storage so far investigated, 10 to 12 per cent. concentrations of carbon dioxide would appear, however, to have exerted an effective control of the growth of *Pencillium expansum,* and to have caused a moderate restriction of the growth of *Sporotrichum carnis* when compared with its growth on the controls stored in air. A quantitative determination of the comparative rates of growth of *Sporotrichum carnis* in air and in 10 per cent. carbon dioxide showed that the average times taken for the colonies grown on Czapek's agar to reach a diameter of 6 mm. were 27 and 36 days respectively, the temperature of storage and the relative humidity being —1°C. (30.2°F.) and 99 per cent. respectively. The corresponding linear expansions, expressed in millimetres per 100 hours, were 1.2 and 0.9. respectively. These results indicate that the rate of growth of *Sporotrichum carnis* in 10 per cent. carbon dioxide is 70 per cent. (approx.) of that in air, an observation closely agreeing with that of Tomkins(10).

(iii) *Changes in Fats.*—The free acidities of various samples of fat, removed immediately after chilling and at the completion of storage, are given in Table IV. In all cases, their "active" oxygen contents did not exceed the equivalent of 0.3 ml. of 0.002 M. sodium thiosulphate, and therefore being extremely small, the values have not been recorded.

TABLE IV.—FREE ACIDITIES OF FATS AFTER CHILLING AND AFTER STORAGE.

Test No.	Sample.	Free Acidity (Per Cent.).					
		After Completion Chilling.	Control Stored 28 Days.	CO_2 Stored 43 Days.	Control Stored 55 Days.	CO_2 Stored 55 Days.	CO_2 Stored 55 Days + 2 Days at 10° C.
1.	Back	0·51	..	2·15	..	..	..
	Abdomen ..	0·53	..	1·36	..	..	..
	Neck + Thorax ..	0·54	..	2·00	..	..	..
	Back, heavy bacterial contamination	..	1·69	..	..	..	..
	Abdomen do. ..	..	2·06	..	..	..	..
	Neck and Breast do.	..	1·95	..	..	..	..
2.	Back	1·53	..	..	2·65	2·02	..
	Abdomen ..	1·24	..	..	1·64	1·24	..
	Neck + Thorax ..	1·50	..	..	1·93	2·07	2·07
	Mixed, heavy bacterial contamination	..	..	..	2·84	..	..
	Mixed, heavy mould (*Penicillium*) contamination	..	..	..	2·45	..	..

Tests of the palatability showed that during storage in 10 per cent. carbon dioxide for a period of 43 days (Test 1), all samples developed stale odours and flavours variously described as "fishy" and acrid. All samples taken at the completion of storage (55 days) in Test 2 had developed slight "off" odours and flavours, but there were detectable differences only in the case of the fat taken from the neck and thorax areas of the air-stored beef, which had a rather more pronounced acrid flavour than that of the corresponding sample from the beef stored in carbon dioxide. Samples taken from quarters held for two days at a temperature of 10°C. (50°F.) subsequent to storage at —1°C. (30.2°F.) for 55 days showed no further development of "off" flavours. The mixed sample from the beef stored in air showing heavy bacterial contamination on the fat had developed an extremely pronounced acrid flavour. In the case of beef stored in carbon dioxide, there were no areas of fat heavily coated with bacterial slime.

The initial mean free acidity of the fat in Test 2 was 1.4 per cent., and at the completion of storage, it had risen to 2.1 and 1.8 per cent. for the air and carbon dioxide stored fats respectively. While the difference between the latter values is probably due to the development of heavy microbial contamination on the air-stored fat, thereby affording a useful index of the effects of microbial proliferation, the presence of the fatty acids alone, as Barnicoat(1) has shown, does not produce "off" flavours. These are probably due rather to attack by bacteria on the connective tissue of the fat than to any marked chemical changes in the glycerides themselves.

(iv) *Bloom of the Meat.*—The loss of bloom of chilled beef is, in some measure, proportional to the extent of the total loss of weight from slaughter to the completion of storage, and it has been found that if such losses exceed 5.5 per cent. (approx.)—the maximum allowable depends largely on the quality of the carcasses—the consequent loss of bloom is serious. In these tests, the losses of weight during periods of 46 and 57 days, from slaughter to completion of storage, have been 2.8 and 4.6 per cent. respectively. Except for the presence of a dull colour of the fat in areas covered with bacterial slime and the occurrence of slight darkening of exposed muscle, the bloom of the meat was not greatly impaired. This result was to be expected in view of the fact that the weight losses were considerably below the critical value. The presence of carbon dioxide did not appear to depreciate the bloom, either by affecting the colour of the fat or by causing a darker colour of the exposed muscles and a greater depth of penetration of methaemoglobin than in similar tissues in the beef stored in air.

(v) *Palatability of the Lean.*—The lean of beef stored in carbon dioxide had acquired a slightly tainted flavour only in exposed areas developing "slime" during storage. This condition, of course, applied only to Test 1.

4. Discussion.

While an insufficient number of experiments have been carried out to enable an accurate assessment to be made of the value of carbon dioxide in prolonging the safe period of storage of chilled beef, it is clear that the use of carbon dioxide will not, of itself, secure freedom from wastage during the period of the transport from Queensland to Great

Britain. It is desirable, therefore, to indicate generally some, at least, of the conditions to be adhered to in order that a trade in chilled beef reasonably free from wastage may be established.

The length of storage possible without the onset of wastage is largely dependent on the nature and degree of the initial contamination of the beef by " low temperature type " micro-organisms. While the maximum numbers of such organisms allowable per unit area of the beef cannot yet be specified, many experiments, not recorded in this communication, have indicated that by strict attention to cleanliness in every phase of the treatment of the beef in the meat-works, it is possible greatly to reduce the degree of contamination normally prevailing. A striking instance of the importance of this factor is revealed by the fact that, in the first and second tests, the ratio of the respective initial " low temperature type " contaminations per unit area of the beef was 10:1, while that of the respective durations of storage until the onset of the " slime " stage was 1:2.5.

Information is available concerning the effects of two factors operating during storage of the beef, viz., the temperature and the composition of the atmosphere. In order to restrict microbial growth, the temperature should be maintained as close as possible to the freezing point of the muscle tissue, —1°C. (30.2°F.). If some ice formation in the meat is not considered undesirable, however, the temperature may, with advantage, be reduced to about —1.5°C. (29.3°F.). For beef obtained from meat-works where the initial contamination consists chiefly of *Achromobacter*, the use of 10 to 12 per cent. carbon dioxide in the storage atmosphere produces an increase of 40 per cent. in the storage " life " of chilled beef compared with storage in air. The greater margin of safety secured, therefore, indicates the desirability of the use of this gas in ships' holds during the prolonged transport from Australia to Great Britain. Haines(5) has indicated that the use of 10 per cent. carbon dioxide almost doubles the time required for the production of a given number of *Achromobacter* organisms at 0°C. (32°F.); this divergence from the results recorded above may possibly be due to differences in the strains of organisms studied.

By experiments now in progress, it is hoped to define the optimum values of the factors responsible for the degree of superficial desiccation during storage, viz., density of stacking of the meat, relative humidity and rate of movement of the air.

In each stage of the treatment and storage in Test 2, conditions extremely favorable to prolonged storage were maintained. Not only was the initial count of micro-organisms extremely low, but the rate of reduction of temperature during chilling, and the degree of superficial desiccation were high. During storage, too, a rate of loss of weight approaching the limit defined in section 3 was employed in conjunction with the maintenance of a constant temperature of —1°C. (30.2°F.) and an atmosphere containing 12 per cent. carbon dioxide. With these conditions prevailing, the period of holding before the onset of appreciable deterioration, including the period at atmospheric temperature equivalent to the duration of the marketing of the

beef, was approximately 60 days. It seems doubtful, however, whether an initial contamination as low as that prevailing in Test 2 can be constantly maintained in large-scale commercial production. While it is difficult accurately to determine the effect of the probable average initial contamination under improved works' technique, it may be assessed tentatively as equivalent to a deduction of seven days from the storage "life" obtaining in Test 2. That is, when storage in carbon dioxide is employed, the probable maximum duration of holding of chilled beef is approximately 53 days, which is equivalent to a period of transport (storage) of 45 days. The latter period is somewhat less than the prevailing average duration of the voyage from Queensland ports to Great Britain.

These observations apply mainly to beef exported from works wherein *Achromobacter* is the predominating species in the initial "low temperature type" contamination. Owing to the fact that carbon dioxide is a relatively effective suppressor of the growth of the more common moulds, except *Sporotrichum carnis,* chilled beef exported from works wherein the former moulds comprise the bulk of the "low temperature type" contamination may reasonably be stored for a considerably longer period than has been indicated in the preceding paragraph.

5. Acknowledgments.

These experiments, and many others now in progress, have been rendered possible through the generous provision of laboratories and equipment by the Queensland Meat Industry Board. The investigators are also indebted to Messrs. Swift Australian Company Proprietary Limited for the loan of many bodies of beef.

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Blue Stain in *Pinus radiata* (*insignis*) Timber.

Some Preliminary Experiments with Case Stock.

*By J. E. Cummins, M.Sc.**

Summary.

Owing to the increased consumption of locally-grown softwoods in Australia, the surface stains, which are caused by fungi and to which such timbers are susceptible, are important, particularly to the Australian case manufacturer.

Experiments aimed at the control of the stains by means of treatment with various solutions are described. Treatment with Lignasan (a proprietary material) effectively prevented blue stain development in case timbers even under bad stacking conditions. However, under such conditions, considerable development of surface mould occurred, but this could be effectively reduced by the provision of better foundations and thicker spacing strips in the stacks.

Treatment with soda solutions reduced blue stain development, but insufficiently for practical purposes. Mould development was also considerable. No experiments were made using improved stacking methods alone. In practice, better results would undoubtedly be obtained if the stacks were so built as to allow of more rapid drying of the timber.

1. Introduction.

Timber, particularly that of coniferous trees (softwoods) is susceptible to stains which may vary in colour from blue, pink, or green, to brown, yellow or black. These stains are either chemical or fungal in origin.

Chemical stains are generally caused by the reaction of some material with constituents of the wood sap, e.g., many Australian hardwoods contain tannin, and when they come in contact with iron, e.g., nails, a blue-black compound causing a characteristic ink-like stain is formed. Chemical stains do not cause serious degrade, and are usually removed when the timber is dressed. They are not further considered in this article.

Fungal stains are essentially different in nature and are of three general types, namely—(i) surface mould stains, (ii) wood-destroying fungus stains, and (iii) sap stains.

Surface moulds are generally green, blue, pink, yellow or black in colour, and are often characterized by a cottony or downy growth. They may sometimes appear powdery in texture, and are often confined to the sapwood only. They do not penetrate the wood and can be easily removed on surfacing. In stock which requires subsequent machining, they are not considered a defect, but in material such as case stock, they may cause serious degrade. In general, moulded cases should not be used for uncovered foodstuffs on account of the possibility of their contamination and subsequent breakdown. The development of surface moulds is often found in the kiln drying of green timber which requires initial conditions of low temperature and high humidity. When such moulds are seen developing in the kiln, they can be effectively killed by subjecting the charge to a steaming or high humidity treatment at 160°F. to 170°F. for one to two hours, the time depending on the effective circulation in the kiln. As well as discolouring the boards, such surface moulds are liable to interefere seriously with the circulation and cause uneven or retarded drying.

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Stains caused by wood-destroying fungi vary in colour. They may be present in the living tree, or they may develop in logs held in the bush or at the mill for long periods before conversion, or in timber stored under bad conditions. In general, they do not occur in yards in which good or relatively good seasoning practices are followed. They will not be discussed further in this article.

Sap staining fungi, as distinct from surface moulds, penetrate the wood and generally cause a blue or brown stain which cannot be removed by surfacing. It is this group of stains which causes tremendous losses in softwoods and minor losses in Australian hardwoods.

Sap stains which are blue in colour are common in hoop pine, kauri pine, bunya pine, and *Pinus radiata* (*insignis*), and some mills are experiencing considerable losses due to their action. Because of the increasing cut of Australian-grown pines, the problem of this sap stain prevention becomes of major importance, and it is proposed to conduct large scale experiments under Australian conditions in order to develop satisfactory methods for preventing or greatly reducing this form of degrade. The present article reports the results of some preliminary experiments on the prevention of blue sap stain in *P. radiata* case stock.

2. Sap Stains produced by Fungi.

Fungi may cause various types of stain particularly on the sapwood. Such stains are commonly bluish or yellow to brown, and different groups of fungi are responsible for the different discolourations caused. The stain which occurs on the sapwood of both hardwoods and softwoods, and which is bluish in colouration, is caused by representatives of several different genera of fungi, commonly called blue stain fungi. In Australia, no detailed investigation of these fungi has been made, but it is known from cultures prepared from infected wood that several species at least are present. Recently, Audrey M. Eckersley(1) has identified two forms closely related to *Ceratostomella pilifera*(Fr.) Winther and *Ceratostomella coerulea* Münch, from *P. radiata* grown on the Mornington Peninsula, Vic., and *Hormonema dematioides* Lagerberg and Melin from material grown at Macedon, Vic. The group of fungi causing the yellowish to brown sap stain in U.S.A., are often species of *Penicillium*, but no investigation of these has been made in Australia. However, some recent investigations have indicated that a species of *Cytospora* is responsible for a yellowish to brown discoloration in Tasmania myrtle (*Nothofagus cunninghamii*) sapwood.

Blue staining fungi chiefly inhabit the sapwood, and apparently feed principally upon the cell contents such as starch, sugar, &c., although some slight breakdown of the cell walls also occurs. As they develop, they penetrate deeper into the wood, and the fine fungal threads or hyphae pass from cell to cell chiefly in the medullary rays or parenchyma tissue. The passage from cell to cell is mainly through the pits or slit-like openings in the cell walls. In parenchyma tissue, more penetration of cell walls appears to take place than in tracheids, where passage from cell to cell is through the bordered pits. Examination of sections of badly blue stained wood shows apparent considerable breakdown of some of the ray cells.

In general, blue stain fungi do not penetrate the truewood,* but Lagerberg, Lundberg, and Melin(2) have recently reported cases where this definitely occurs. However, the extent of degrade due to truewood attack appears to be relatively small.

The newly-formed fungal hyphae are colourless, but after a few days they become coloured, and when blue stained wood is examined under the microscope, the hyphae appear yellowish-green to brownish-green in colour, although their effect is to cause the wood to appear bluish. Under certain conditions, and when the fungus has developed in the wood, its fruiting bodies can be seen as small black specks on the surface of the timber.

The conditions for the development of blue stain fungi are very similar to those necessary for the growth of other wood inhabiting fungi, particularly wood destroying varieties, but their effect on the timber is essentially different. For instance, they have only a slight effect on the strength of the timber, and, for general use, blue stained timber should not be rejected in the same way as that affected by wood-destroying fungi.

As conditions of development are similar, excessively blue stained material should not be accepted under conditions where strength is important, as typical decay fungi may also be associated with the blue stain. Further, blue stain generally indicates the presence of sapwood, and under conditions where resistance to decay is desirable, sapwood is not durable unless previously effectively treated with wood preservatives.

For their maximum development, blue stain fungi require food, moisture, air, and warmth. Food is provided by the sapwood of the tree, and ordinary weather conditions are suitable for development, although warm moist weather, as experienced particularly in northern New South Wales and Queensland, is conducive to rapid growth. Experiments by numerous investigators have shown that blue stain will readily develop at moisture contents of the sapwood (based on the oven-dry weight of the wood) ranging from about 35 per cent. to 120 per cent.(2), while growth may occur as low as 23 per cent. and as high as 150 per cent. The air requirements of the fungi are very small, but it is possible that growth at high moisture contents is limited chiefly on account of the restricted air supply due to complete filling of the cell cavities with sap.

Because of the ability of the sap stain fungi to develop satisfactorily over such a wide range, all timber is susceptible to attack at some time in its history. Blue stain is found in the living tree, the log, and the converted timber. In the living tree, infection is due to mechanical bark abrasion or the action of bark-boring or wood-boring insects which carry the spores on their bodies and spread the infection. Bark beetles and associated blue stain have been shown by Craighead(3) to cause the killing of pine trees in the United States of America. Logs left in the bush are susceptible to attack, particularly in warm moist seasons, and blue stain is frequently found extending from the end of logs and from places where the bark has been broken. The rapid removal of logs from the bush and their immediate conversion will do

* The term "truewood" has been adopted to describe what is usually termed heartwood. In Australia, the central portion of a tree is very often affected by decay or has little strength. This portion, which is really part of the heartwood, is called "heart." The terms "heart" and "heartwood" are therefore confusing, and that portion of the tree between the "heart," or the pith, and the sapwood has been named the truewood.

much to reduce the bluing. The use of sprays or end coatings is also effective at times, but this aspect of the problem needs much further experimental work before definite recommendations can be made. Sawn timber is particularly susceptible to attack, but efficient methods of piling can do much to control stain development. The obtaining of a dry surface by suitable provision for rapid initial drying and the maintenance of this dry surface is the basis of stain control during air seasoning. Kiln-drying of timber green from the saw will obviously reduce losses, but with certain classes of stock this is not commercially possible, and all mills are not equipped with kilns. Chemical dip treatments, as discussed later, are a further effective means of stain control.

Once timber has been infected, the spread of the stain may be very rapid. During periods of warm moist weather, it may completely penetrate the sapwood of logs or boards within a few days or weeks (see Plate 1).† Material bulk stacked when green can become seriously degraded within several days.

It appears that the slightly acid condition of the wood sap is particularly favorable for fungus development. The use of alkaline dips such as soda are based on this theory, the dipping rendering the surface of the wood alkaline. However, as soon as the alkali is more or less neutralized, staining is liable to occur if other conditions are suitable. Lignasan, a proprietary article* stated to contain 0.43 per cent. of ethyl mercury chloride, has recently been introduced into the timber industry in the United States of America as a sap stain preservative, and experiments carried out on both an experimental and commercial scale by various workers of the U.S. Bureau of Plant Industry(4, 5, 6, 7) have shown that good results have been obtained from its use. Sodium carbonate and sodium bicarbonate have both been used extensively for preventing sap stain, and, before the use of newer chemicals in the last few years, formed one of the commonly accepted standard treatments.(8) However, they have not given consistently good results, particularly under bad conditions of bluing.

Chemical dips are intended primarily to protect the timber during the early stages of drying and until the surface is below the moisture content at which stain will develop, but, even with their use, good piling practices are also essential.

Once timber has been dried below the danger point, it is not immune from further attack if it is allowed to become wet again, for the causal fungi can remain dormant for long periods pending the time when moisture conditions are again suitable for their growth.

3. Experimental Work in Australia.

Pinus radiata is at present largely used in Australia for case stock, but such stock is often cut at small mills utilizing hedge-grown material. In consequence, the shooks often receive little care in handling and are frequently delivered to the case manufacturer bundled green. However, conditions for their rapid drying are not always available to the manufacturer and air-drying space is often restricted. Under such circumstances, the development of blue stain all too frequently results.

† See facing page 308.
* Manufactured by the E. I. du Pont de Nemours Co.

One case manufacturer in Melbourne approached the Division with reference to prevention of blue stain which was occurring in his factory and causing serious losses. An inspection of the factory showed that conditions for blue stain development were very favorable, and, as the proprietor was agreeable to make a limited amount of material available, it was decided to initiate preliminary experiments.

Outline of Experimental Work.—At the factory, case shooks are received from various small mills on the Mornington Peninsula, Vic., the green stock being received within a few days of cutting. On receipt, the shooks are "lap" stacked* on the second floor of the factory. The practice was to build stacks right on to the floor, each stack two boards wide and about 6 feet high, leaving a central space in each stack of from 7 to 11 inches, the space depending on the width of the material stacked. Case sides were used for end strips. Spaces between stacks averaged about 18 inches. The floor has doors at each end, but, except when a strong north wind is blowing, the air circulation throughout the room would be very poor and irregular. Stacks were erected both parallel and at right angles to the direction of air flow between the doors.

It appeared that there were two ways by which improvement could be effected. Firstly, much could be done in the way of a more efficient stacking practice by the provision of suitable foundations, the use of thicker strips, and the laying out of stacks so as to take advantage of all the available air circulation. Secondly, the use of chemical dips with only slightly modified piling practice could be attempted; and it was decided to conduct the experiments with these and with modifications in stacking.

Materials for the Treatments.—A load of case sides (approximately 1,500 boards) of *P. radiata,* 21 x 7 x ⅜ inches, were made available for the work. They were cut at Red Hill, Vic., the day before treatment.

For treating solutions, it was decided to use (*a*) Lignasan† and (*b*) a mixture of sodium carbonate and sodium bicarbonate. The Lignasan solution was made by dissolving ½ lb. of dry powder in 20 gallons of cold water, and the soda solution by dissolving 14 lb. of washing soda and 6 lb. of bicarbonate of soda in 20 gallons of water (the solution thus being equivalent to 3 per cent. of soda ash and 3 per cent. of soda bicarbonate).

Method of Treatment.—Before treatment, each board was carefully examined for the presence of stain, and when this was found it was marked. Most of the stain found was due to saw marks and handling. Treatment was carried out by completely immersing the boards in the treating solution for at least five seconds. The boards were then allowed to drain for a few minutes and were then placed in their respective stacks. The Lignasan treatments were made with cold solution, but the soda solution was maintained at 140 deg. F.

Erection of Stacks.—The experimental stock was stacked in different ways. Three stacks—one Lignasan treated, one soda treated, and one untreated control—were erected, using a "lap" stack slightly modified from that in use at the factory. The modifications consisted of the

* An example of "lap" stacking is shown in Fig. 1 (top).

† In Australia, Lignasan costs about 60 cents U.S. currency per lb., and treatment would thus probably cost for chemicals alone only about 2d. per 100 super. feet of case stock.

addition of 4-in. foundations and the substitution of $\frac{3}{8}$-in. square strips at the ends, instead of the factory practice of using case sides for end strips (see Fig. 1 (top)).

Two stacks—one Lignasan treated and one untreated—were erected on 4-in. foundations, each tier of boards being separated by $\frac{3}{4}$-in. square strips (see Fig. 1 (bottom)).

FIG. 1.—(*Top*)-Diagrammatic sketch of "lap" stack. Strips 3/8″ thick. Foundations 4″ high.

(*Bottom*)-Diagrammatic sketch of "strip" stack. Strips 3/4″ thick. Foundations 4″ high.

In both types of stacks a central flue of 7 inches was left. Stacks were three boards long, two boards wide, and were all approximately 4 feet high, and there was a space of 2 feet between stacks. Dummy stacks were erected in order to ensure that the end stacks did not receive comparatively more air circulation than the others, and also to simulate factory conditions more closely.

Period of Test.—Periodical cursory examination of the stacks was made, and it was found that within three weeks blue stain had developed to a considerable extent. Mould growth was apparent in the soda and Lignasan treated stacks at the same time. All stacks were allowed to remain for a period of about nine weeks, when they were demolished and the shooks examined.

Method of Inspection and Recording of Results.—All stacks were demolished in the same way, boards being removed from each tier commencing from the end nearest the wall and working towards the front. In each tier (or approximate tier in the case of "lap" stacking) there were six boards; each board was carefully examined, and where blue stain or surface mould was present an estimate was made of the percentage surface area affected on both the top and bottom of the boards. From the figures so obtained, the total percentage surface area affected was calculated. In some cases, boards containing small percentages of truewood were noted, the blue stain apparently affecting the total sapwood present. The blue stain in such boards was noted as a percentage of the area of the whole board surface. Exact differentiation of sapwood and truewood was difficult, and it did not appear necessary to record such detailed results, the actual difference on the whole being negligible.

The moisture content of four boards in each five tiers was determined electrically (by a Blinker), and the average of these taken as the average for the five tiers.

Results of Test.—In Table 1 the results of the detailed analyses of the untreated and Lignasan treated "lap" stacks are given, and in Table 2 the summarized results of all stacks. The moulds reported are due to the growth of a green-coloured *Penicillium.* The effect of faster drying is shown in the reduced stain or mould development in the top and bottom tiers of the stacks.

Considering the "lap" stacks, it is seen from Table 2 that 71 per cent. of the total surface area of the boards in the control stack was blue stained compared with 40 per cent. in the soda treated and none in the Lignasan treated. With the exception of one board, no mould growth occurred in the untreated stacks, whereas it was common in both the soda and Lignasan treated stacks. In the soda stack, it occurred alone in the top boards, but lower in the stack it was intimately associated with blue stain. The development of mould in the Lignasan stack was about equal in percentage to the blue stain development in the soda treated boards. Soda treatment reduced the total percentage of stain and also the percentage number of boards affected, the latter from 93 to 69.

A study of Tables 1 and 2 and the detailed results not shown indicated that, with increasing depth in the stack, there occurred a greater development of stain and mould which was closely correlated with the average moisture content determined at the time of examination. In the top fifteen tiers of the soda-treated stack only one board was slightly stained, while in the untreated stack 78 boards were stained, although the average moisture contents were very similar. Soda, therefore, retards blue stain development, but only under certain conditions. It is suggested that the value of soda solutions as a stain preventative may be a function of the time; that is, soda treatment will prevent blue stain spores from germinating or developing for a limited time only, corresponding to the period required for the neutralization of the alkali by acids diffusing out from the interior of the timber.

The development of mould growth both on Lignasan and soda treated boards is also probably due to the hydrogen-ion concentrations on the surface of the boards, this being such that it is at, or near, an optimum for their development. Also, it is evident from a comparison of Lignasan and untreated "lap" and "strip" stacks that a high moisture content for a considerable time is more necessary for mould than for blue stain development; the success of Lignasan for preventing both blue stain and mould is definitely associated with the method of stacking and the provision in the surface layers of a drying rate sufficiently fast to reduce the moisture content below the minimum required under the conditions created by the Lignasan solution. The latter solution is definitely alkaline. The pH on the surface of the wood, which is normally acid, is thus increased, and in the case of *P. radiata* it is suggested that the increase in pH produces suitable conditions for mould spore germination and subsequent development. A study of the pH of wood and of the change with time in pH of Lignasan and soda dipped boards, when stacked under conditions retarding drying, would give interesting data, and would probably result in the development of suitable solutions to prevent both mould and blue stain development.

TABLE 1.—VARIATION OF BLUE STAIN, MOULD, AND MOISTURE CONTENT IN UNTREATED AND LIGNASAN-TREATED "LAP" STACKS.

No. of Tiers from Top of Stack.	Blue Stain (as percentage of area).		Mould. (as percentage of area).		Average Moisture Content when Unstacked.	
	Untreated.	Treated.	Untreated.	Treated.	Untreated.	Treated.
1–5 ..	20	Nil	Nil	0·1	17	17
6–10 ..	55	,,	,,	0·1	19	18
11–15 ..	81	,,	,,	1·3	23	18
16–20 ..	72	,,	,,	28	24	21
21–25 ..	72	,,	,,	30	>30	25
26–30 ..	61	,,	,,	53	>30	28
31–35 ..	91	,,	,,	65	>30	>30
36–40 ..	85	,,	,,	64	>30	>30
41–45 ..	90	,,	,,	59	>30	>30
46–50 ..	89	,,	,,	73	>30	>30
51–55 ..	55	,,	,,	72	26	>30
56–60 ..	..	..	..	28	..	25
Average ..	71	Nil	Nil	39	30 (approx.)	30 (approx.)

All figures on basis of susceptible boards.

In the untreated stack all susceptible boards were affected with blue stain with the exception of 12 out of 30 in the top five tiers and 9 out of 30 in the bottom five tiers.

In the Lignasan-treated stack all susceptible boards were affected with mould with the exception of 29 out of 30 in the top five tiers; 23 out of 30 in the second five tiers; 23 out of 30 in the third top five tiers; 8 out of 28 in the fourth top five tiers; and 5 out of 12 in the bottom five tiers.

TABLE 2.—SUMMARIZED RESULTS OF EXAMINATION OF TREATED AND UNTREATED STACKS.

Type of Stack.	Treatment.	No. of Boards Susceptible.	Blue Stain (as percentage of area).	Mould (as percentage of area).	No. of Boards Affected.	Percentage Boards Affected.	Average Moisture Content when Unstacked.
"Lap"	Untreated	310	71	Nil	289	93	30
	Soda ..	336	40	Mould associated with blue stain	231	69	30
	Lignasan	312	Nil	39	224	72	30
"Strip"	Untreated	220	31	Nil	148	67	20
	Lignasan	250	Nil	1·7	37	15	20

The provision of $\frac{3}{4}$-in. spacing strips reduced the percentage area blue stained from 71 per cent. to 31 per cent., and the number of boards affected from 93 per cent. to 67 per cent. in the untreated stack, and the percentage of mould in the Lignasan stack from 39 per cent. to 1.7 per cent. and the percentage of boards affected from 72 per cent. to 15 per cent. By the provision of foundations at least 12 inches high and the use of 1-in. spacing strips, the trouble from moulding of Lignasan-treated boards would probably be eliminated under the conditions in the factory storage room. The mould in the "strip" Lignasan stack was only comparatively light in development and the degrade of

the affected boards therefore not serious. The provision of foundations and the use of 1-in. spacing strips as suggested above would probably not prevent blue stain development in untreated stacks under the existing storage conditions.

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Footrot in Sheep.

*By D. Murnane, B.V.Sc.**

The work, the results of which are given in the article that follows, forms part of the programme of investigations that is being undertaken by the Council's Division of Animal Health under the Australian Pastoral Research Trust—Empire Marketing Board scheme. (See this *Journal*, Vol. 4, August, 1931, p. 133). The author has kindly been accommodated at the Veterinary Research Institute, Parkville, of the University of Melbourne. In addition, some of the pens used by him were erected at the Zoological Gardens, Melbourne, by arrangement with the authorities concerned.—Ed.

1. Introduction.

This disease constitutes a major problem involving much field and laboratory work which has engaged our part time attention for the past two years. From the very nature of the condition, it is obvious that the investigation is one which presents considerable difficulties, the chief of which are the complications caused by the gross bacterial contamination of the lesions. It is of considerable economic importance, not on account of high mortality in affected flocks, but because of the loss of condition of affected animals and the cost of constant treatment. In addition, its existence in a flock takes shillings per head off the sale value.

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Footrot is an acute, sub-acute, or chronic disease affecting the feet of sheep and occasionally cattle. It occurs in most countries of the world, and has been known for very many years, being described in Europe as early as 1791. In Australia, it is more prevalent in certain districts than in others, and often assumes epizootic proportions.

Predisposing Factors.—Certain conditions such as overgrown hooves, wet or damp pastures, and heavy growth of pasture (particularly clovers) have been noted to favour the development of footrot, but none of these can be credited with causing the disease. It is a generally accepted fact that the finer-woolled sheep are more susceptible than the coarser breeds, but it is doubtful if any breed is resistant.

Types.—The clinical picture of footrot is well known to sheep-owners in affected districts. We recognize two more or less distinct types—(*a*) the ordinary "sub-acute" form which frequently passes to a chronic state; and (*b*) the less common "acute" or "malignant" type.

Symptoms.—In the first-mentioned form, the infection commences with a reddening of the skin between the digits, accompanied by a hot condition of the affected foot—due to the inflammatory process which is taking place. This phase is frequently referred to by sheep-owners as the "scald stage," and is usually accompanied by lameness. As yet, there is no visible break in the skin. Later, there is some swelling of the affected tissues and an oozing of clear serous fluid. The area becomes quite moist and develops a characteristic and unmistakable offensive odour. Subsequently, the skin breaks down and pus forms. The horny structures become under-run with pus, and as the vascular soft structures (sensitive laminae) beneath the horn are attacked and digested, the soles and even the walls are shed. When only the sole is shed, the wall tends to spread and turn outwards and upwards, leaving the sensitive structures exposed and obviously causing extreme lameness to the extent of forcing the animal to move about on its "knees." This state may be reached in the comparatively short time of two weeks, but usually takes longer. The lesion may progress slowly or may remain at a standstill (neither improving nor becoming worse) for months. This constitutes what we term the "chronic" state.

Throughout the progress of the disease, the affected animal usually loses weight considerably, partly on account of the pain suffered, partly on account of the inability to move about and obtain sufficient food, and doubtless on account of considerable toxic absorption from the foul lesion.

Occasionally, when making post-mortem examinations on footrot cases which have died, we have found multiple abscess formation in the liver, lungs, and spleen, and it seems likely that these originated from the foot lesion. Usually, however, the lesions are confined to the feet, below the coronet.

The "acute" or "malignant" type, on the other hand, runs a somewhat different course. The lesion seems to commence either in the pastern or in the fetlock joint above the foot, and for a time remains localized in this region. There is considerable swelling of the affected joint with much pain. The tendons and ligaments in the vicinity are

the structures which are particularly attacked. They are rapidly digested, often resulting in luxation of the extremity. Sinuses usually form, and there is a foul discharge, or the pus burrows downward and the foot itself becomes involved. The animal being forced to move about on its "knees," it frequently develops lesions in the knee joints and in the region of the sternum ("brisket") through inoculation of abrasions by contact with discharging lesions in the lower joints. This type of footrot generally runs a more rapid course and is more severe, not infrequently terminating in the death of the animal. (The disease in cattle commonly resembles this "malignant" form in that the tendons and ligamentous structures are involved, but it seems to clear up more readily than in sheep.)

Differential Diagnosis.—There are few diseases of the feet of sheep which may be mistaken for footrot, particularly in Australia, where, fortunately, foot and mouth disease does not exist, but, even in countries where it is prevalent, the differentiation should not be difficult when the disease has progressed past the initial stage.

There is, however, an abnormal condition of the foot not infrequently encountered in sheep running on wet or swampy country which closely resembles the early stage of footrot, and with which it may easily be confused. There is maceration, erosion, and even suppuration of the interdigital tissues accompanied by marked lameness. This condition readily yields to treatment, and in fact may even clear up spontaneously, especially if affected animals are removed to dry surroundings. It is often referred to by farmers as the "non-infectious type."

The writer is of the opinion that both types are infectious, and that both originate in the same manner (i.e., by infection of abrasions of the skin of the foot), the progress of the condition and the ultimate lesions depending entirely on the type and virulence of the invading organism.

Natural Infection.—There is little doubt that the disease is infectious. The nature of the lesion and the rapidity with which the trouble spreads through a flock when introduced under favorable conditions seems to adequately support this view. It is quite common to find owners who state that they never experienced footrot prior to the purchase of a few infected animals from which the disease rapidly spread to their flocks. That infection may take place quite rapidly is demonstrated by the fact that young lambs of affected ewes have been known to contract the disease within two or three weeks of birth.

Etiology.—The determination of the causal organism has presented the greatest difficulty. In the case of an infectious disease affecting an internal organ, the isolation of the infecting germ is usually a simple bacteriological procedure, as the organism is as a rule uncontaminated by others. In the case of footrot, we have an external lesion in contact with soil, faeces, &c., and consequently contaminated with hundreds of organisms—pathogenic and non-pathogenic. This makes the task of determining the initial causal organism infinitely more difficult. Certain workers in France have attributed footrot to infection with the tissue parasite *Treponema podovis* which they claim to be present in enormous numbers in the lesions. This view has been supported by one or more workers in America, although as far as we are aware the disease has not been produced experimentally by inoculation with this organism, nor has the organism even been cultivated.

Other American workers (notably Mohler and Washburn) are equally firm in the opinion that the causal organism is *Bacillus necrophorus.*

After microscopical examination of a large number of smears and preparations from affected feet, we strongly incline to the latter view. We have found that in practically 100 per cent. of cases examined, *B. necrophorus* is present, while in only two or three cases* have we seen organisms resembling the *Treponema* mentioned, and, further, it is very questionable whether these were of a pathogenic nature. However, at present we refrain from stating that the *Treponema* is of no significance. Whether we are justified in incriminating *B. necrophorus* as the primary causal agent of footrot may be open to doubt. Owing to the unbiquitous character of the organism, it may be that it is a common secondary invader. But, whether it be primary or secondary, the fact remains that the chief lesions seen in the vast majority of cases of footrot (neglecting the mild non-progressive type mentioned earlier) are definitely those of necrosis associated with the presence of *B. necrophorous.*

B. necrophorus is not credited with penetrating unbroken or healthy skin; and we believe that in the case of footrot this probably holds true, the tissues of the foot being first injured or abraded by some other agent, either traumatic, climatic, parasitic, or bacterial, thus permitting the entry of the organism.

The Organism.—As part of the Australian investigations of footrot, *B. necrophorus* has been studied in detail by Messrs. H. R. Carne and W. I. Beveridge, at the F. D. McMaster Laboratory in Sydney, and has been described very fully by the latter in a recent paper which will shortly be published. The more general characters may be found in any modern bacteriological textbook. Suffice it to say that the germ is a filamentous, non-sporing, highly pathogenic anaerobe, and is regarded as a common inhabitant of the normal intestine of pigs (and probably ruminants and horses), of faeces, and of soil contaminated with faeces.

Pathogenicity.—The number of disease conditions with which *B. necrophorus* is associated is surprising. It is capable of producing lesions in various parts of the body in most of the lower animals and in man. Rabbits and mice are particularly susceptible, and are therefore commonly used for the purpose of isolating the organism from natural footrot lesions.

Emulsions of scrapings from the deeper parts of the lesion (at the junction of the diseased and healthy tissues) are injected subcutaneously or intramuscularly. A necrotic lesion results and extends rapidly as the organism multiplies and attacks adjacent healthy tissue. Death of the inoculated animal usually takes place within 10 days. The extension of the necrotic area is so rapid that most of the original contaminating organisms (many of which are non-pathogenic) are left behind at or near the site of inoculation. Necrotic material from the periphery of this lesion is then taken and injected into a second experimental animal.

* Two of these cases were received from the Division of Animal Nutrition, Adelaide, through the kindness of the Chief and Mr. Lines.

After two or three such passages it is usually possible to recover *B. necrophorus* in pure culture, or so slightly contaminated that it can be isolated by cultural methods such as growth in deep solid media. Some workers inject the contaminated material intravenously into rabbits, in which case necrotic lesions develop in the liver. We prefer the subcutaneous or intramuscular method.

Pathology.—In footrot, the natural lesion seems to be essentially of a necrotic nature. There appears to be progressive death of the sensitive structures of the foot, followed, of course, by bacterial liquefaction. From the position of the lesion, this is to be expected, as there is every opportunity for heavy contamination from the soil and faeces. But in the deeper regions of the lesion, near the junction of healthy and affected tissues, *B. necrophorus* appears to be the predominating organism, and in fact may often be seen in almost pure culture.

2. Experimental.

A large amount of experimental work with sheep has been carried out, of which a general outline of the tests made, with results, is given below.

(i) *Tests to prove the infectious nature of the condition.*

(*a*) A series of tests was set up whereby groups of clean sheep were placed in close contact with groups of chronic natural cases in small pens, where they remained for a period of four months. Control groups of equal size were held in adjoining pens under similar conditions, except that they were not in contact with affected animals. As this experiment was carried out during the dry months, the soil was kept moist by daily irrigation. *Result:* No footrot developed in the contacts (or in controls).

(*b*) The above tests were repeated during the winter months, using the same sheep and the same pens. *Result:* All contacts developed footrot. Controls remained free. The experiment was repeated, using different pens. *Result:* Contacts developed footrot. Controls free.

(ii) *Effect of maceration alone.*

(*a*) Several groups of clean sheep were kept standing in water for approximately 8 hours daily for a period of several weeks. *Result:* No footrot.

(*b*) Numbers of sheep were fitted with canvas boots ("footrot boots") which were daily filled with water for a period of several weeks. *Result:* No footrot.

(iii) *Direct inoculation with pus.*

Numerous groups of animals have been lightly scarified between the digits and smeared with pus from natural cases. Early in the investigation, these tests were attended by negative results. Later experiments on similar lines, however, have given positive results in the majority of cases, particularly when the animals were kept under wet conditions.

(iv) *Inoculation with filtrates of material from natural lesions.*

In order to determine whether or not a filtrable virus is associated with the condition, the filtrate from an emulsion of diseased material which had passed through a bacteria proof (L2) filter was smeared on scarified areas on the feet of several groups of clean sheep at different times. *Results:* Negative in all cases.

(v) *Inoculation with pure cultures of individual organisms isolated from natural lesions.*

A number of different anaerobes were isolated and cultured. Separate sheep were scarified and inoculated with each organism, *Results:* Negative.

(vi) *Inoculation with cultures of B. necrophorus.*

Numerous groups have been lightly scarified and inoculated with pure cultures of this organism. *Results:* Most of the earlier tests were negative. Later experiments gave inconsistent results. More recent tests have given positive results in the majority of cases.

(vii) *Inoculation with culture of B. necrophorus isolated from natural case of bovine footrot.*

Two sheep and one calf were scarified and smeared with culture. *Results:* Definite and typical lesions developed in all three animals.

(viii) *Test to ascertain whether early cases of footrot will progress if the subjects are removed to thoroughly dry surroundings.*

Several very early natural cases were brought from the country and placed in a perfectly dry pen at the laboratory, where they were retained for 6 weeks. Footrot progressed to an extremely advanced stage, the hooves being shed in two cases.

3. Treatment.

The first step in the elaboration of any curative or preventive measures is to establish the cause, and most of our effort has been spent in this direction. On account of the numerous requests from sheep-owners for advice as to treatment, it was felt that something might be done in the meantime, even though such treatment were more or less empirical and only partially successful. Several methods of treatment and numerous proprietary preparations have been tested. As a result, we offer the following recommendations, which, we hope, may be improved upon as our work progresses.

Preventive.—On properties where footrot is known to occur, the feet of all sheep should receive careful attention and should be closely watched. It is unnecessary to stress the point that prevention is better than cure and less costly. Overgrown feet should be trimmed, and, if infection is suspected, all animals should be passed through a suitable footbath once a week. Where the country is wet or where pasture is rich and heavy, beneficial results have been reported from the practice of providing small plowed areas here and there which form comparatively dry "camps" for the animals. (It may be noted how affected sheep themselves frequently seek dry spots such as banks of dams, on which to camp.)

Curative.—The first essential is prompt action. Only too often the first few lame sheep in a flock are neglected until a large number have become affected. At the first sign of trouble, the flock should be yarded. The visibly affected animals should be isolated, while the remainder should be passed through a footbath with the object of prevention.

All affected animals should be kept isolated in a small convenient paddock and receive the following intensive treatment:—The affected feet should be thoroughly trimmed; all loose, under-run or separated horn should be removed; and the animals should then receive prolonged footbath treatment. On a property where footrot is prevalent, a well constructed and adequate footbath is an absolute necessity. The owner who tries to cope with footrot without a footbath is usually wasting his time and money. A concrete bath, in the form of either a race or a pen capable of accommodating 50 sheep or more according to the size of the flock, should be constructed. The solution to be used is a matter of choice. Numerous proprietary preparations are available, in respect to the efficacy of many of which very extravagant claims are made by the manufacturers. However, it can be stated that most of these products will give results if used in the manner about to be described, as will agents such as copper sulphate (bluestone), formalin, and "Monsol." The strengths recommended are—

Copper sulphate—5% solution (= 1 in 20, or ½ lb. to the gallon of water).

Formalin—2% solution.

Monsol—1% solution.

Having pared the affected feet, the animals are placed in the footbath solution. They are not simply walked through the bath and out at the other end as is the common practice, but are held in the solution for at least one hour. The reason for this is obvious. It allows the antiseptic to penetrate the sinuses and pockets of pus in the affected foot. A mere walk through the bath does nothing more than wash the exposed surfaces, and no matter how efficient an antiseptic may be, it clearly cannot be effective if it is not permitted to come in contact with the organisms against which it is being used. While it may suffice as a preventive, unsatisfactory results must always follow mere walking of animals through a footbath as a curative method.

Therefore, we stress the point that the length of time the affected feet are allowed to remain in the footbath is a most important factor. It is for this reason that we have recommended a footbath of large capacity. In the case of affected sheep, the treatment should be repeated several times at two-day intervals. We have been able to demonstrate that extremely advanced (chronic) cases readily recover under this intensive treatment.

Similar results can be obtained by the use of "footrot boots" which are on the market. The canvas "boot," filled with an antiseptic solution, is placed on the affected limb for a period of several hours. Where a large number of animals are to be treated, the use of a footbath would be preferred by most owners.

Bluestone is a cheap and effective solution, but it has a disadvantage in that it stains wool. Formalin in 2 per cent. solution is even cheaper than bluestone 5 per cent. It is quite effective, and does not stain wool.

In addition, it has a desirable hardening effect on the affected feet. Where possible, sheep should be held in a shed or on a dry floor for several hours after footbath treatment. When animals are kept for a prolonged period in a footbath, the solution becomes fouled with droppings which float and which should be skimmed off after use each time. This adds to the length of life of the solution. We find that the same solution can be used 4 or 5 times at two-day intervals before having to be discarded.

The question of local (external) dressings applied after trimming the affected feet has received consideration. Although the antiseptics are usually employed in more concentrated form in these cases, we are of the opinion that such dressings cannot be relied upon to give satisfactory results, on account of the impossibility of getting the antiseptic into all small pockets and sinuses. From the numerous preparations used, one of the following may be selected:—

1. Picric acid—4 ounces .. } Apply with a brush.
   Methylated spirit—1 gallon }

2. Powdered resin—2½ lb., dissolved in 1 gallon of turpentine, then add "Monsol"—½ pint. Shake or stir vigorously until a uniform mixture is obtained. Apply with a brush.

It will be found that, as it dries, this second mixture forms an adhesive coating on the foot, thereby holding the antiseptic in contact longer than would otherwise be the case.

3. Formalin—1 part, glycerine—9 parts.

4. A mixture recommended by the Victorian Department of Agriculture, made as follows:—

   Stir into 1 quart of warm Stockholm tar 2 ounces of finely ground bluestone, and add 1 tablespoonful of lysol or "Monsol." Apply with a brush.

The use of powerful irritants such as pure carbolic or hydrochloric acid, pure formalin, &c., is condemned on account of resulting destruction of healthy tissue.

The "malignant" or joint type of infection is much more difficult to deal with. It is best treated by surgical methods, i.e., opening the "pocket" of pus with a clean sharp knife, removing as much as possible of the necrotic tissue, and syringing out the cavity with an antiseptic solution such as hydrogen peroxide 1 part, water 3 parts. The pus "pocket" may then be packed with the following antiseptic powder:—Boracic acid and chlorinated lime (bleaching powder), equal parts.

In this type of infection, the destruction of tendons and ligaments is frequently so extensive that permanent deformity of the joint remains.

The treatment for cattle is similar to that recommended for the "malignant" type in sheep, and could be combined with footbath methods.

Further work is in progress, by which it is hoped to confirm our tentative conclusions and to elaborate more effective curative and preventive methods. The question of a preventive vaccine is being kept in view, although the nature of the disease probably does not offer great possibilities in this direction.

Downy Mildew (Blue Mould) of Tobacco.

I. The Influence of Over-Wintered Plants, II. Wild Hosts, and III. Spraying.

By A. V. Hill, B.Agr.Sc., and H. R. Angell, Ph.D.*†

Summary.

1. Over-wintering diseased tobacco plants were shown by microscopical examination and field observation to be sources of seedbed infection by downy mildew.

2. *N. glauca*, systemically infected with downy mildew, was found growing in close proximity to seedbeds even in districts remote from tobacco-growing areas.

3. Spraying seedlings with 2–2–40 Bordeaux mixture did not prevent the occurrence and spread of downy mildew when the disease was epidemic in neighbouring seedbeds.

4. Downy mildew did not occur early in isolated seedbeds, nor was it destructive where the plants were grown under relatively dry atmospheric and soil conditions.

1. Over-wintered Plants.

Introduction.—The widespread occurrence of downy mildew in the tobacco-growing districts of New South Wales and Victoria during the autumn of 1932 led to the expectation that many diseased plants would survive the winter months and produce new growth bearing conidia to initiate and spread the disease in the spring. Since but little definite experimental work was previously done with the object of showing to what extent over-wintered diseased plants might be the source of outbreaks in seedbeds in commercial tobacco-growing areas, an attempt was made in 1932 to determine their importance under those conditions.

On account of the absence of wild hosts of the downy mildew fungus and because of its relative nearness to the research laboratories at Canberra, the Tumut district was selected for the work.

Importance of Over-Wintered Plants.—Diseased plants growing in the experimental plots at Canberra during 1930 were examined from time to time, and areas bearing conidia were found on them in autumn. Microscopic examination at that time showed that mycelium was present in the leaves, stems, and roots. The aerial portions of the plants were, as usual, killed by frosts during the winter, but the basal portions remained alive, and the dormant underground buds began growing during the early part of September. Some of the shoots were removed before they reached the level of the surface of the soil, and. after being sectioned and stained, were examined under the microscope. The mycelium of a coenocytic fungus was found growing intercellularly in the tissues of the young shoot. In the following month,

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other shoots on the same plants, which were allowed to grow above ground, showed the characteristic symptoms of the disease. It was evident that downy mildew survived the winter as mycelium in the plants, and that the disease on the new spring growth arose from it and not from external sources.

The production of conidia on over-wintered plants in farmers' fields was repeatedly observed during the past four years. The importance of such plants as a source of infection of seedlings in spring was recorded by Adam(1) and the writers(2).

Further evidence presented in this paper serves to emphasize the necessity for eradication and destruction of tobacco plants immediately harvesting is completed.

Cultural Practice in Relation to Over-Wintering Plants.—The bulk of tobacco grown in New South Wales and Victoria at present is produced on river flats which are subject to flooding during the winter months. If such fields are cultivated during autumn and winter, the occurrence of floods, usual in spring, may result in a considerable amount of soil erosion. Tobacco fields, therefore, are often left uncultivated until a few weeks before they are required for the following season's crop. Under such conditions, the majority of the plants from which the leaf has been harvested remain alive during the winter months of June, July, and August. Some of the plants are killed by flood waters, but others remain alive and may even produce leaves throughout the winter, the young shoots being protected from slight frosts by the old or dry leaves overhanging them. In most cases, however, the aerial portions of the plants are killed by frosts, but the crown and roots remain dormant until the following spring, when new shoots are produced. Fields of such plants are commonly seen in tobacco-growing areas during September, October, and November.

As seedbeds are sown in July, August, and September, the main sowings being completed in August, and transplanting is done during November and December, the danger of infection from over-wintered plants is evident.

According to Dickson(5), flood waters in New South Wales and Victoria during the winter of 1931 covered practically all the land planted to tobacco in the previous season, the over-wintered plants being killed thereby. The absence of such plants, the use of clean seed, and a comparatively unfavorable season for downy mildew, resulted in late development of the disease instead of early in the season as is usual. Consequently, a plentiful supply of seedlings was available for the 1931-32 season.

As the disease is more or less severe each year in the areas where the main crops are grown, Victorian growers endeavour to sow seedbeds in dry localities far removed from such districts. The extension of commercial tobacco production to the drier seedbed areas during the 1931-32 season resulted in the establishment there of over-wintering plants.

During the spring of 1932, it was consequently difficult to find an area suitable for seedbeds that was also remote from over-wintering plants and accompanying tobacco diseases prevalent during the previous season. Under the circumstances, the seedlings were likely to be

attacked by diseases which were present on plants in the district. A survey of the areas concerned showed that this factor was one of those responsible for outbreaks of downy mildew during the 1932-33 season.

Observations in the Tumut area in 1932.—While conducting spraying experiments in the Tumut district during the spring of 1932, old tobacco stalks were located, and observations made on the dates of appearance of the disease on them and subsequently in the nearby seedbeds.

In this area, tobacco is grown on river flats in narrow valleys surrounded by mountainous country which isolates the district from other agricultural areas. While on visits to the fields in the autumn of 1932, it was observed that plants in many of them were seriously affected with downy mildew. On 16th August, another examination showed that many plants from the previous season were still alive and producing new growth. In one field, the lower surfaces of some young leaves measuring 5 inches in length, were covered with conidiophores and conidia of the downy mildew organism. Seedbeds were actually being sown in the district at that time. Three weeks later, over-wintering diseased plants were still present in the fields, and at the same time, seedlings were developing in the seedbeds throughout the district.

As the conidia were seen on the leaves of the over-wintered plants as early as 16th August, early infection in seedbeds was expected. On 18th October, other over-wintered plants with a profuse development of conidia were found, and within 300 yards was a seedbed that had downy mildew. In the seedbed, the disease was not on scattered individuals but on groups of plants. Seedlings from the same lot of seed, sown half a mile further from the over-wintered plants than those just mentioned, did not become diseased until 14th November, i.e. 26 days later. On 30th October, the disease also attacked other seedlings nearer the over-wintered plants but in the opposite direction.

An attempt was made to trace the spread of the disease throughout the district, but owing to the disinclination of many farmers to admit so early in the season that it was then present, it was impossible to carry out the plan. Infection was reported from a number of seedbeds two weeks after the above-mentioned outbreak of 18th October.

The experimental seedbeds sown during the first week of September were situated at intervals of approximately ⅓ mile from over-wintered diseased plants. On 29th October, disease was observed in the nearest one ⅓ mile away, on 9th November in another ⅔ mile away, but the seedlings at a distance of 1 mile remained healthy until 28th November. This may be taken as indicating that the disease spread in ever-widening circles from the over-wintered plants and from the earlier attacked seedbeds, the number of conidia in the meantime increasing from the relatively few thousands on the original plants to countless millions produced in each seedbed every day. Those seedlings farthest removed remained healthy for the longest time, provided the organism was not introduced into the seedbed on the clothes and implements of workmen.

The association of over-wintered diseased plants with the initial outbreak and subsequent spread of downy mildew to seedbeds definitely shows the importance of such plants as sources of infection.

2. Wild Hosts.

Introduction.—In a previous publication(2), the writers briefly discussed the host plants and the history and geographic distribution of of the downy mildew fungus. It was shown by inoculation experiments that about twenty, and therefore probably all, species of the genus *Nicotiana* including *N. glauca* Graham and *N. suaveolens* Lehm., which are widespread in Australia, are susceptible to downy mildew. The importance of these plants as carriers of the disease is more than is at present realized by tobacco growers. Both species probably occur in all the States of the Commonwealth.

N. glauca—Tree Tobacco.—The comparative drought resistance of *N. glauca* was perhaps one of the reasons for its use as a hedge plant and as an ornamental shrub in gardens many years ago. Later, it escaped from cultivation and became established in many localities. It now occurs near Charters Towers, Queensland, is fairly common in dry districts north of Adelaide, South Australia, and has been observed by the writers at Tamworth in the north and Deniliquin in the south of New South Wales, in the Murray River Valley from Corowa to Cobram, at Shepparton in the Goulburn Valley, and also near Sea Lake and Melbourne, Victoria. In many cases it occupies comparatively small areas near townships, but in other localities it is spread over large areas, and it would therefore be difficult, if not impracticable, to eradicate it. Because *N. glauca* is considered a poisonous plant, it has been declared a noxious weed in many shires of New South Wales(6). The plant may therefore be a source of loss both to tobacco growers and stock-owners. (Plate 2.)*

N. suaveolens.—Another species of wild tobacco, *N. suaveolens,* is regarded by some as native to Australia. Bailey(3) records three varieties, *longiflora, parviflora,* and *Debneyi* in Queensland. Black(4) records *N. suaveolens* and *N. excelsior* in various localities in South Australia. McAlpine(7) observed downy mildew on *N. suaveolens* in Victoria, and this plant was reported in 1932 by Pittman(8) as a host of downy mildew in Western Australia. According to Stewart(10), it caused the death of some bullocks near Narrabri, New South Wales, in 1908, and that it was poisonous to sheep was also shown by Seddon and McGrath(9). It has not been reported from Tasmania. This plant, therefore, like *N. glauca,* is both poisonous to stock and a host for downy mildew of tobacco. Its occurrence in northern New South Wales may possibly be correlated with the appearance of downy mildew in tobacco, for it was in this area that the disease is commonly believed to have been first seen in Australia.

Both *N. glauca* and *N. suaveolens* tend to be restricted to localities having an average rainfall of about 25 inches or less. At Deniliquin and Cobram, in 1932, the former was severely attacked by downy mildew, the young shoots being in some cases killed by the disease. Conidiophores and conidia were seen on the affected leaves. Nearby tobacco seedbeds were seriously attacked. As this plant is a perennial shrub, the mycelium of the fungus is able to persist from year to year in the stems, from which it grows into the leaves and produces conidia by which the disease is spread to tobacco seedbeds.

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* See Plates facing page 308.

It is therefore essential for growers to ensure that neither *N. suaveolens* nor *N. glauca* occurs near the site of the seedbed. If eradication is impracticable, another site should be selected in an area where neither wild hosts nor over-wintering plants occur.

3. Spraying Experiments.

(i) *Introduction.*—Bordeaux mixture is the fungicide most extensively used for the control of downy mildews other than that on tobacco. This spray material gives a useful measure of control of some diseases, if the foliage can be readily wetted by it, but as some of the leaves of tobacco are close to, and parallel with, the ground, wetting their lower surfaces with spray is a difficult matter. Since infection of tobacco by downy mildew takes place at any stage of growth, and on any part of the surface of the leaf, it is necessary from shortly after germination to cover both the upper and lower leaf surfaces with spray.

Nevertheless, the spraying of isolated seedbeds may possibly be of some value, as the number of conidia present in the atmosphere around remote seedbeds would be fewer, and the plants would be given partial protection by the fungicide.

The spraying experiments conducted at Tumut and Canberra by the writers(2) during 1931 did not give promise of an effective means of control of downy mildew, but more experimental work was considered necessary before recommendations were made. As previous experiments showed that repeated applications of 4–4–40 Bordeaux were harmful to seedlings, a 2–2–40 mixture was used.

(ii) *Tumut Experiments.*—At Tumut, in 1932, a series of five seedbeds, each approximately 20 square yards in area, was set out at intervals of about $\frac{1}{3}$ mile in a section comparatively isolated from other seedbeds. Seed of the variety Warne was sown on 8th September. The seedbeds were covered with butter muslin until the seedlings were established, about 4-5 weeks after sowing. Spraying with 2–2–40 Bordeaux mixture was begun on 15th October when the plants were three weeks old, and was continued at five-day intervals, one-half of the seedbed area in each case being sprayed and the other half left as a control.

On 17th October, downy mildew was first reported from a farmer's seedbed about $\frac{1}{3}$ mile distant from experimental seedbed No. 1. Several diseased plants were present in the unsprayed section of seedbed No. 1 on 29th October, and 16 days later the disease had spread throughout both sections. At the time the first infection was observed, the leaves of the largest seedlings were $\frac{1}{2}$ inch in diameter. Infection became general throughout both the unsprayed and sprayed seedlings before the plants were fit for transplanting. The protection afforded by the spray was therefore of no practical value.

Diseased plants were first seen on 9th November in the unsprayed section of seedbed No. 2, which was approximately $\frac{1}{3}$ mile distant from seedbed No. 1 and $\frac{2}{3}$ mile from over-wintered diseased plants. In the sprayed section, the disease was seen 15 days later. During the interval, one lot of plants suitable for transplanting was taken from the sprayed

bed. In this case, therefore, the spray gave useful protection for 1 to 2 weeks. More effective protection might have been given by spraying all the beds.

Seedbed No. 3, situated 1 mile from the over-wintered diseased plants, was in a sunny position on well-drained, sandy soil, such a location being deemed comparatively unfavorable to the onset and spread of downy mildew. On 28th November, diseased plants were found in both the sprayed and unsprayed sections of this seedbed. Previous to this date, two lots of plants were taken for transplanting. The disease spread comparatively slowly and was less severe in the sprayed section.

Downy mildew was also found in seedbeds 4 and 5 on 28th November, the disease appearing in sprayed and unsprayed sections of seedbed 4, and in the unsprayed section of seedbed 5. It was found in the sprayed section of the latter seedbed, 9 days later. Seedlings were obtained from both these seedbeds before downy mildew occurred, and an extra lot was obtained from the sprayed section of seedbed 5.

The location of the seedbeds in relation to over-wintered diseased plants is shown on the accompanying plan (Fig. 1), while the occurrence of downy mildew in the seedbeds is shown in Table I.

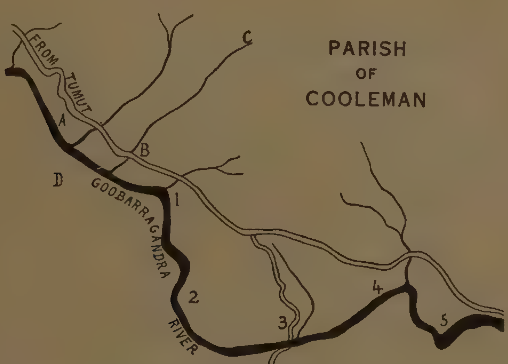

Fig. 1.—Showing the location of experimental seedbeds, referred to in the text as Nos. 1–5, in relation to over-wintered diseased plants at A. The earliest reported outbreak of downy mildew in the district was in seedbeds at B., and the site of other seedlings, which remained healthy for a longer period, is shown at C. The latter were from the same seed as was sown at B. The seedbeds at D. were also diseased.

Discussion.—In these experiments, the protection afforded seedlings by Bordeaux mixture varied from 0 to 16 days. As the disease attacked some of the seedlings when they were too small for transplanting, the protection of 16 days was, in this instance, of no practical value, but from the sprayed sections of two other groups of seedbeds one extra lot of seedlings was obtained.

In each group, the unsprayed section was adjacent to the sprayed. Therefore, as soon as the disease attacked either section, sufficient conidia were produced to infect all plants in the seedbed, in spite of the fungicide. On account of the mode and rapidity of growth of seedlings, complete protection by sprays or dusts is very difficult, if not impossible, to obtain in practice.

Table I.—Occurrence of Downy Mildew in Sprayed and Unsprayed Seedbeds at Tumut, 1932.

Seedbed Number.	Distance from Over-wintered Plants showing Disease on 18th October.	Occurrence of Disease in Seedbeds.		Protection by Spraying.	Usefulness of Spray.
		Unsprayed.	Sprayed.		
1	$\frac{1}{3}$ mile	29 Oct.	15 Nov.	16 days	None. Seedlings too small for use as transplants
2	$\frac{2}{3}$ mile	9 Nov.	24 Nov.	15 days	One picking of plants from sprayed seedbed
3	1 mile	28 Nov.	28 Nov.	None	None
4	$1\frac{2}{3}$ miles	28 Nov.	28 Nov.	None	None
5	2 miles	28 Nov.	7 Dec.	9 days	One extra picking of plants from sprayed bed

The seedbeds were attacked in the order of their distance from the original focus of infection, the earliest attacked being those nearest the diseased, over-wintered plants. It appears, therefore, that isolation from sources of infection was of more practical value than the use of the fungicide. Many instances have been brought to our notice of isolated seedbeds that remained disease-free for long periods, sometimes until after the transplanting season was over. In other instances, they were infected by persons who had previously been handling diseased plants. The importance of man as an agent in introducing the disease was further emphasized by the writers' experience at Canberra, where research work on downy mildew was in progress under glasshouse and field conditions. On three successive occasions, the disease was purposely introduced, and after each general infection, all diseased seedlings were destroyed and fresh sowings made after intervals of several weeks. Unless the disease was deliberately introduced by us, seedlings remained healthy, even though conditions were most favorable for its development. It was

necessary in every case to obtain diseased material from tobacco-growing districts in order to infect the first plants in the glasshouse or field. From these infected plants, the disease spread to all others in the glasshouse. After the disease was introduced, the only method of eradicating it was by the destruction of all tobacco seedlings and plants.

In tobacco-growing areas, eradication of diseased tobacco seedlings is impracticable, because that procedure may involve the loss of the year's crop, but eradication of unused seedlings and of plants after harvesting involves no loss, while the time and money spent in doing it is an investment that will minimize loss in the following seasons.

The use of clean seed, and freedom from diseased over-wintering plants throughout an entire district, would very materially assist in the production of healthy seedlings throughout the season, or, if the disease appeared, its chances of causing serious loss would be lessened by its delayed appearance.

(iii) *Canberra Experiments.*—Four seedbeds, each 18 square yards in area, separated by distances of approximately 25 yards, were sown in the experimental plots of the Council for Scientific and Industrial Research at Canberra on 22nd August. Spraying with 2–2–40 Bordeaux mixture was commenced on 21st October, and was continued at five-day intervals on three of the seedbeds. The other seedbed served as a control. Towards the end of October, it was found necessary to introduce downy mildew from the Tumut district, situated about 70-80 miles away in a westerly direction. Seedlings in the glasshouse, about 100 yards distant from the seedbeds, were inoculated with the introduced material, and became infected. The disease spread rapidly in the glasshouse, and from the glasshouse to the unsprayed bed, in which it was seen on 22nd November. In the two nearby sprayed seedbeds, it appeared on 2nd December, and in the most distant sprayed seedbed on 12th December. The experiment was then discontinued.

On account of the presumably unfavorable climatic conditions at the time, the disease did not spread rapidly even among the unsprayed seedlings, and only comparatively few seedlings in the sprayed beds were attacked.

Discussion.—In experimental work during the past four years it has been found that climatic conditions at Canberra (high temperature, low rainfall and humidity) are comparatively unfavorable to downy mildew during the late spring and summer months. Control measures based on experiments in this or other areas with a similar climate may not be effective when applied in districts where the climate is different and the disease is in consequence epidemic every year.

Two sprayed seedbeds remained free of disease for 10 days, and the third seedbed for 20 days after the unsprayed seedlings were attacked, but the disease did not spread very rapidly either in the sprayed or unsprayed seedbeds. On the other hand, the disease was epidemic in similar seedbeds at Tumut at about the same time. Climatic factors, therefore, were probably more important than the spray in the control of the disease. In Tumut, seedbeds on a well-drained sandy soil in a sunny position were less seriously attacked.

In commercial tobacco areas, therefore, more attention should be given to choosing a seedbed site that is well drained, in a sunny position, and if possible on sloping land, to allow of adequate air drainage. These precautions will tend to delay the attack and rapid, destructive spread of the disease.

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Addendum.

An examination of the tobacco-growing districts and seedbed areas of southern New South Wales and Victoria early in September of this year (1933) showed that conidia were being produced in abundance on over-wintered plants which were present wherever tobacco was grown during the previous season. In many cases, such plants were found within a few yards of seedbeds in which the seedlings were still young.

Foot and Root Rots of Wheat in Australia.

The Influence of the Combined Action of *Fusarium culmorum* (W.G. Sm.) Sacc. and *Urocystis tritici* Koern. on the Occurrence of Seedling Blight.

*By W. L. Geach, B.Sc.**

Summary.

1. During the past three seasons, an unusual amount of seedling blight was observed under glasshouse conditions among plants grown in unsterilized soil from grain inoculated with spores of *Urocystis tritici.* Isolations from the affected plants yielded *Fusarium* spp., generally *F. culmorum*, and *Helminthosporium sativum*, or both.

2. In an adjoining glasshouse, plants grown from grain inoculated with *F. culmorum*, or in soil inoculated with *H. sativum*, were not so consistently nor severely attacked by seedling blight as those in the flag smut experiments referred to above.

3. When grain was inoculated with a mixture of spores of *U. tritici* and conidia of *F. culmorum*, a greater amount of seedling blight occurred than among plants from grain inoculated with either of these organisms alone, whether in the field or in pots containing sterilized or unsterilized soil. In contrast with this, a comparatively small amount of seedling blight occurred under the same environmental conditions among plants from grain inoculated with only *F. culmorum* or *U. tritici.*

4. Under field conditions, varieties highly resistant to *U. tritici* were only comparatively resistant to seedling blight due to the combined attack of the two organisms.

5. The two organisms acting together under ordinary field conditions are partly responsible for poor stands.

6. It is possible that *U. tritici* or other smuts, in combination with other foot rot pathogens, may yield results similar in general effect to those reported in this paper. Preliminary experiments indicate that this is the case with *H. sativum* and *U. tritici.*

1. Introduction.

During the years 1930-33, an unusually large amount of seedling blight consistently occurred among wheat plants that were being grown in an unheated section of the glasshouse for testing varietal resistance and susceptibility to flag smut. The soil was unsterilized, and of a dark, sandy, alluvial type, and the inoculum consisted of flag smut spores with which the grain was coated. At and above the soil-line, typical symptoms of seedling blight appeared. The coleoptiles changed from the normal green to brown, the leaves yellowed and withered, and the plants died after the bases of the stems were rotted through. Sometimes, the aerial mycelium of a species of *Fusarium* could be readily observed around the bases of the affected plants. The symptoms appeared on seedlings up to the time when they were about 4 inches high. Those passing that stage continued apparently normal growth until later on when symptoms of flag smut usually appeared.

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Isolations from the blighted seedlings usually yielded species of *Fusarium,* generally *F. culmorum* (W.G. Sm.) Sacc., or *Helminthosporium sativum* P.K.B. or both.

During the same period, the writer was engaged in the study of the factors influencing the degree and amount of seedling blight and other manifestations of foot rot caused by *F. culmorum.* In experiments specifically designed for this purpose, such consistently good infection as that in the flag smut trials above noted was not obtained, even though the plants were grown in sterilized soil, from seed inoculated with a heavy suspension of conidia, and under environmental conditions considered optimal for the disease.

On comparing the results in the two glasshouses, it was quite evident that a most important factor was influencing the amount of seedling blight in the flag smut experiments. That factor is shown by the experiments detailed in this paper to be the combined action of a smut and a root-rotting organism, in this case, *U. tritici* and *F. culmorum.*

2. The Organisms.

Fusarium culmorum (W. G. Sm.) Sacc.

In a previous paper(9), the writer showed that *F. culmorum* is one of the causal organisms of foot rot of wheat in Australia. Material from 87 fields out of 190 inspected during the 1932 survey of part of the Riverina, Mallee, and Wimmera districts, yielded cultures of species of *Fusarium* of the "discolor" type. A number of the isolations, representative of the districts visited in the course of the survey, were tested under glasshouse conditions, and about 90 per cent. of them were found to be pathogenic on wheat. Judged by the macroscopic appearance and pathogenicity of the isolations, they were cultures of *F. culmorum.*

The inocula used in the experiments reported in this paper were conidial suspensions from sub-cultures of the isolation from the wheat variety "Purple Straw" originally obtained from Longerenong, Victoria. This culture was selected on account of its abundant production of sporodochial conidia and its relative virulence.

Urocystis tritici Koern.

Flag smut of wheat caused by *U. tritici* Koern occurs in Australia, Japan, India, the United States of America, S. Europe, S. Africa, and China.

According to Hori(12), McAlpine(14), and Brittlebank(3), *U. tritici* is seed-borne. Verwoerd(21) reports that the spores easily adhere to the grain and are found in great numbers in the crease and brush. It is also obvious from the results of experiments that they are present in the soil in areas where flag smut occurs, and may persist in it for five years or more.

Noble(17), Verwoerd(21), and others have attempted to culture *U. tritici* on various artificial media, but without success. In the present state of our knowledge, it must therefore be regarded as an obligate parasite.

Infection by *U. tritici* is possible only during a limited period, stated by Noble(17) to be during the time when the coleoptile is less than 4 mm. in length at inoculation, and by Verwoerd (21) as being from the emergence of the coleoptile to its rupture by the first leaf.

The inoculum* used in the experiments recorded here was obtained from plants growing under field conditions, and it was therefore naturally expected that spores of other organisms were likely to be present. Under the microscope, it was observed that the samples of inoculum contained spores of *Alternaria* and a few rust spores, the former occurring very much more frequently. From time to time, platings were made, totalling altogether about twenty. Cultures of only *Alternaria* spp., *Penicillium* spp., and bacteria resulted.

3. Materials and Methods.

(i) *Grain.*—The varieties selected for pot experiments were Federation and Bomen, the former classed as relatively suspectible to, and the latter relatively resistant to, flag smut. Before inoculating and planting, the grain was washed in running tap-water for fifteen minutes. The controls were sown without further treatment, whereas other lots were inoculated by dipping them in heavy suspensions of spores of *U. tritici*, or of sporodochial conidia of *F. culmorum*, or a mixture of both, as required. The grain was sown immediately following treatment.

(ii) *Soil.*—A dark, sandy, alluvial soil obtained from a nearby river valley was used in all pot experiments, either with or without previous sterilization by steam. For those experiments in which sterilized soil was used, 6-in. pots were filled, and then steamed for four hours or more at atmospheric pressure.

4. Results of Experiments in the Glasshouse.

In all but one of the experiments, 100 grains of the selected wheat variety were sown in pots at the rate of 10 grains per pot for the control and for each of the inoculated sets. On completion of germination, and subsequently at intervals of two weeks, the seedlings were counted, and the numbers of dead plants visible were also recorded. The latter are not given in the accompanying table. As previous experience showed that whiteheads were rarely produced in pot experiments in the glasshouse, probably no useful purpose would have been served by allowing the plants to mature. Since any attempts to assess the amount of damage done by root or foot lesions involved a certain amount of error and difficulty, it was decided that, in this series of experiments, death due to the disease would be the most suitable standard to employ. In two experiments, the plants that survived the seedling stage were allowed to grow almost to maturity, and then examined. However, the majority of those in the mixed inoculum set had no lesions on the roots nor on the bases of the stems. About one-tenth were smutted, and the others were more or less affected with foot or root rot.

* The flag smut inoculum was very kindly supplied by Miss P. H. Jarrett of this Laboratory, who is at present investigating certain aspects of that disease.

Table 1 gives the results of five experiments with Federation wheat in sterilized soil, one with the same variety in unsterilized soil, and another in soil used in a previous experiment and then repeated, using the soil as the sole source of inoculum for the second experiment. Tests with the relatively resistant variety Bomen are included. The table shows that the trials were made over a period of six months, during which the temperature of the glasshouse varied considerably. The prevailing temperature seems therefore to have been a modifying rather than a limiting factor in these experiments. It follows that the results of the different experiments, as presented in the tables, should be considered separately.

TABLE 1.—SHOWING THE AMOUNT OF SEEDLING BLIGHT RESULTING FROM THE USE OF DIFFERENT INOCULA UNDER GLASSHOUSE CONDITIONS.

—	—	Maximum stand of apparently healthy plants. (A)	Final stand of apparently healthy plants. (B)	Observed dead. (A)–(B)	Per cent. stand based on final stand in controls.	Period of observation and average glasshouse temperatures.
Federation wheat sown in sterilized soil; 5 experiments = 450 grains	Control ..	425	406	19	100	May—14·4°C–24°C
	F. culmorum ..	422	388	34	95	June—12·2°C–22·2°C
	U. tritici ..	431	389	42	95	July—11·3°C–19·9°C
	Mixed inoculum	408	299	109	73	
Federation wheat sown in unsterilized soil; 1 experiment=100 grains	Control ..	97	90	7	100	
	F. culmorum ..	97	90	7	100	21st Mar.–23rd June.
	U. tritici ..	99	93	6	103	
	Mixed inoculum	96	83	13	92	
Bomen wheat sown in sterilized soil; 3 experiments = 240 grains	Control ..	203	203	0	100	
	F. culmorum ..	194	157	37	77	
	U. tritici ..	215	187	28	90	27th July–28th Aug.
	Mixed inoculum	195	131	64	64	
Federation wheat in soil originally sterilized for use in a former experiment with *F. culmorum* and *U. tritici* and used again without re-inoculation of grain	Control ..	199	199	0	100	
	F. culmorum ..	167	144	23	72	4th July–14th Aug.
	U. tritici ..	187	172	15	86	
	Mixed inoculum	156	91	65	45	

The mixed inoculum produced a much higher percentage of seedling blight than either organism acting independently, the distinction being more marked in sterilized than unsterilized soil. Certain factors other than temperature, as above noted, which interfered with quantitative replication of results, are being investigated. Experiments with a mixed inoculum consisting of *H. sativum* and *U. tritici* in sterilized soil under glasshouse conditions have so far given results paralleling those above, but sufficient data to warrant presentation of figures are not yet available. No seedling blight was observed, but a number of seedlings showed lesions, which in many cases extended along the sheaths of the

lower leaves. Other lesions were observed on the laminae. In contrast with this, at no examination so far made of the plants in this experiment, and only very occasionally in the course of other experiments during the past three years, were lesions noticed on stem bases or leaves of plants arising from grain inoculated with *H. sativum* only.

Isolations from the stem and leaf lesions of the infected plants yielded cultures of *H. sativum*.

5. Pot Experiments Out-of-Doors.

It is well known that out-of-doors a more nearly normal type of growth is obtained, even in pot experiments. That was not, however, the original purpose in trying the first of this series outside, which was to observe the effect of the disease at a lower temperature than could be obtained in the glasshouse. The results are shown in Table 2. The disease was not expected in the

Table 2.—Showing the Amounts of Seedling Blight Resulting from the Use of Different Inocula in Pots Out-of-Doors.

—	—	Maximum stand of apparently healthy plants. (A)	Final stand of apparently healthy plants. (B)	Observed dead. (A)–(B)	Per cent. stand based on final stand in controls.	Period of observation and temperatures.
Federation wheat in sterilized soil; 1 experiment = 100 grains	Control ..	96	96	0	100	5th May—21st July
	F. culmorum ..	96	89	7	92	5th May—18th = 14 days after planting, av. temp. 3°C–17°C av. temp. June.—4·7°C–13·3°C; av. temp. July.—8·6°C –15·5°C
	U. tritici ..	100	94	6	98	
	Mixed inoculum	94	44	50	46	
Federation wheat in unsterilized soil	Control ..	98	98	0	100	14th June–28th Aug.
	F. culmorum ..	95	93	2	94	
	U. tritici ..	93	91	2	92	
	Mixed inoculum	98	89	9	90	
Bomen wheat in sterilized soil	Control ..	100	100	0	100	14th June–7th Aug.
	F. culmorum ..	96	92	4	92	
	U. tritici ..	97	96	1	96	
	Mixed inoculum	94	61	33	61	
Bomen wheat in unsterilized soil	Control ..	97	97	0	100	30th May–15th Aug.
	F. culmorum ..	97	95	2	98	
	U. tritici ..	95	95	0	98	
	Mixed inoculum	96	89	7	92	
Federation wheat in sterilized soil. After a fortnight in the glasshouse the pots were removed to a bench out-of-doors	Control ..	185	178	7	100	26th June–3rd Aug. Average temperature in glasshouse after planting 12°C –20°C
	F. culmorum ..	193	175	18	98	
	U. tritici ..	185	180	5	101	
	Mixed inoculum	173	113	60	63	

virulent form obtained in the comparatively warm conditions prevailing inside, because the optimal temperature for infection by *F. culmorum* is from 18°C.-22°C. On several occasions in May, the temperature outside rose to about 20°C., but was not maintained for more than an hour or two, even during the hottest part of the day. On the other hand, in the same month, it was often only just above freezing-point at 9 a.m. According to records of soil temperature made during May, the average minimum and maximum, taken at 9 a.m., and noon at a depth of 1 inch, was 3°C. and 17°C. respectively. Lower average temperatures prevailed during the succeeding winter months, when a number of frosts occurred at night. The average soil temperatures, also taken at 1 inch at 9 a.m. and midday respectively in June, were 4.7°C. and 13°C., and in July, 8.6°C. and 15.5°C. Watering was not required as often as in the glasshouse, since occasional showers and heavy dews contributed to the supply of soil moisture.

It will be observed from Table 2 that the trend of the results is essentially the same as in Table 1, in that the percentage stand in pots which were sown with grain treated with the mixture of the two organisms was much lower than in the other sets (Plate 3).* Seedling blight was produced more slowly by the mixed inoculum in unsterilized soil than in sterilized soil.

From the diseased plants in the mixed inoculum sets, cultures of *F. culmorum* were repeatedly obtained.

6. Field Experiments.

Four plots, each measuring 48 feet x 12 feet, separated by 4 feet pathways, were prepared for preliminary field trials in May, 1933. The soil of the experiment area was a light-coloured, silty loam, somewhat deficient in humus, and was used for experimental work with *F. culmorum* during the previous season. Plot No. 1, used as a control, was sown with uninoculated grain, No. 2 with grain inoculated with spores of *U. tritici*, No. 3 with *F. culmorum*, and No. 4 with a mixture of both these organisms. Twelve varieties of wheat were chosen for trial, viz., Canberra, Federation, Aussie, Caliph, Gluyas, and Waratah, reputedly susceptible to flag smut, and Bomen, Bunyip, Cedar, Galgalos, Geeralying, and Nabawa, classed as resistant. Rows of resistant and susceptible varieties alternating were planted 1 foot apart, each with 39 grains at intervals of 4 inches.

The original purpose of the tests was to compare the effects of the three inocula on the plants at maturity, but during an examination made in June, when the plants had begun to tiller, it was evident that the organisms in the mixed inoculum plot were already causing an appreciable and unexpected amount of seedling blight.

No records of soil temperature were taken at the depth at which the grain was sown, i.e., approximately 1½ inches, but it may be judged from the average minimum and maximum air temperatures and the soil temperatures at 4 inches below the surface. During the first fortnight following planting, the average minimum and maximum temperatures of the air were 5.5°C. and 14.7°C. In May, the average minimum and maximum temperatures of the soil at 4 inches were 8.9°C. and 11.7°C., in June 5.6°C. and 8.3°C., and in July 5.6°C. and 7.8°C.

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* For Plates see facing page 308.

In the accompanying table, the stand of each variety in the control plot on 22nd June and 1st August, is compared with those in the three inoculated plots. The percentage stands, on 1st August, of plants of each variety in the mixed inoculum plot are calculated on the basis of the number in the controls. The total numbers of plants standing on the stated dates, and the percentage stand in each plot on 1st August, are also given. A striking feature in this table is the large number of

TABLE 3.—SHOWING THE AMOUNT OF SEEDLING BLIGHT IN SUSCEPTIBLE AND RESISTANT VARIETIES UNDER FIELD CONDITIONS.

Varieties.	Date of Examination.	Control Plot.		Flag Smut Plot.		Mixed Inoculum Plot.		*F. culmorum* Plot.		Percentage Stand in Mixed Inoculum Plot.
		Apparently Healthy Plants.	Dead.*	Apparently Healthy Plants.	Dead.*	Apparently Healthy Plants.	Dead.*	Apparently Healthy Plants.	Dead.*	
Bomen (R) ..	(*a*)	138	..	123	..	100	4	130	3	
	(*b*)	132	..	118	..	84	1	128	..	63
Canberra (S) ..	(*a*)	140	..	130	..	110	9	131	..	
	(*b*)	137	..	120	..	74	3	130	..	54
Bunyip (R) ..	(*a*)	132	..	130	..	94	1	126	..	
	(*b*)	131	..	120	..	83	2	121	..	63
Federation (S) ..	(*a*)	147	..	141	..	89	8	134	1	
	(*b*)	138	..	119	..	63	..	127	..	45
Cedar (R) ..	(*a*)	134	1	137	1	112	1	129	..	
	(*b*)	124	..	137	..	98	1	126	..	79
Aussie (S) ..	(*a*)	107†	1	132	..	108	6	133	..	
	(*b*)	100	..	113	1	75	1	128	..	56
Galgalos (R) ..	(*a*)	135	1	134	..	117	5	137	..	
	(*b*)	129	..	122	..	97	..	137	..	75
Caliph (S) ..	(*a*)	131	1	138	..	95	3	128	..	
	(*b*)	131	..	118	3	71	4	119	..	54
Geeralying (R)..	(*a*)	133	..	139	..	100	1	122	1	
	(*b*)	124	..	129	..	86	2	113	..	69
Gluyas (S) ..	(*a*)	124	..	139	..	107	20	128	1	
	(*b*)	121	..	120	2	71	6	115	..	58
Nabawa (R) ..	(*a*)	141	2	135	2	105	3	133	1	
	(*b*)	124	..	124	..	88	3	125	..	70
Waratah (S) ..	(*a*)	136	..	138	1	74	4	132	1	
	(*b*)	132	..	128	..	57	1	127	..	43
Totals on 22nd June ..		1,598		1,616		1,211		1,563		
Totals on 1st August ..		1,523 = 100% stand		1,468 = 96·4% stand		947 = 62·2% stand		1,496 = 98·2% stand		

(*a*) = 22nd June. (*b*) = 1st August.

* These plants were dead at the time of counting, but had not yet rotted away.

† Only three rows of this variety were sown as controls. In all the others there were four rows.

dead plants seen and counted in the mixed inoculum plot as compared with the scattered few in the others on the day the plants were counted.

As the first count was made on 22nd June, 43 days after planting, it is logical to assume that the low stand in the mixed inoculum plot was due to pre-emergence blight and seedling blight caused by the two organisms.

The plots were not arranged with a view to statistical analysis of the results, but comparison of the stands of each variety in the mixed inoculum with the control plot on 1st August shows striking differences. The varieties classed as susceptible or resistant to flag smut appear to be also relatively susceptible or resistant to the mixed inoculum. It was observed at each examination that the plants in the control and in the *F. culmorum* plots were about the same height, whereas those in the flag smut and mixed inoculum plots were definitely stunted.

7. Discussion.

That *F. culmorum* is an independent parasite on wheat, causing seedling blight and other symptoms of foot and root rots, has been shown by Appel (1), Bennett (2), and the writer (9). Simmonds (20) has also found that it is parasitic on oats, on which it causes similar symptoms. Although the pathogenicity of this and other foot rot organisms has been proved, no adequate explanation based on experimental evidence in the glasshouse or in the field has hitherto been offered to account for variation in the extent and intensity of their attacks in the same or in different fields from year to year.

Experiments recorded in this paper show that when grain is inoculated with spores of both *F. culmorum* and *U. tritici*, the percentage of seedling blight under glasshouse or field conditions is much higher than that produced by *F. culmorum* alone.

McKinney (15), McKinney and Davis (16), and Dickson (5), found that soil temperature and moisture greatly influenced the amount and severity of foot rot caused by *H. sativum, Ophiobolus graminis* (Berk.) Sacc., and *Gibberella saubinetii* (Mont.) Sacc., respectively. Although in controlled temperature experiments, foot and root rotting has been produced by *F. culmorum* over the whole range tried, i.e., 10°C.-30°C., the optimum temperature for seedling blight appears to lie between 18°C. and 22°C. when the soil moisture is adjusted to 60% of its capacity. In pot experiments in sterilized and unsterilized soil out of doors, seedling blight occurred among plants in the mixed inoculum sets when the average temperature varied from 4.7°C. to 13.3°C. Furthermore, in the mixed inoculum plot in the field, seedling blight was observed when the average soil temperature range was 8.9°C.-11.7°C. in May, 5.6°C.-8.3°C. in June, and 5.6°C.-7.8°C. in July, whereas there was practically none in the adjoining plot in which the grain was sown after being inoculated with *F. culmorum* only. It is clear from these figures that when grain is inoculated with the two organisms, seedling blight occurs abundantly in the field or in pots out-of-doors at lower temperature than would ordinarily be expected from the results of controlled temperature experiments with *F. culmorum*. According to Faris (7), the amount of flag smut depends on both soil temperature

and moisture. He found that between 10°C. and 20°C., and with fairly dry soil, a high percentage of infection was obtained. When the soil moisture was 40%, high infection occurred only when the grain was germinated between 10°C. and 15°C. If the soil moisture approached saturation, extensive infection occurred at 10°C., but dropped rapidly above and below that point. In our work, when grain is inoculated with both *F. culmorum* and *U. tritici,* a greater amount of seedling blight results over a wider range of temperature, irrespective of whether sterilized or unsterilized soil is used in the experiment.

Fellows (8), Henry (11), and Broadfoot (4) report on the marked difficulty in producing cereal foot rots in field experiments, even when the soil is heavily inoculated, and other conditions so far as they are known, are satisfied. Henry (10), and Sanford and Broadfoot (19), suggest that the loss in virulence of *H. sativum* and *O. graminis,* respectively, in field experiments, is due to the controlling or suppressive action of the soil microflora. Attempts by the writer and others in this laboratory to produce well-marked foot rot symptoms by artificial inoculation of the soil in experimental field plots have not been altogether successful, and in certain cases have almost entirely failed to produce a large amount of rotting. On the other hand, it is commonly known that sometimes the disease occurs in severe form under field conditions. The results reported here provide a partial explanation of such attacks, the variation in the amount and severity being perhaps correlated with soil temperature and moisture during germination. The occurrence of thin stands or even stunted plants such as are frequently found in areas affected with foot rot may be in great measure due to joint attack by one of the smuts and one or more of the foot rot organisms. Experiments are now in progress with other smuts, in combination with *F. culmorum* and other foot rot organisms of wheat, barley, and oats.

That the manifestation of a disease caused by one organism may be intensified as a result of the action of another has been noted by other investigators. Dillon Weston (6) in experiments on the control of bunt, observed that smutted wheat plants were more heavily attacked by *Puccinia glumarum* Eriks. & Henn., than were healthy plants. Welsh (22), found a similar relationship existing among oats, in that smut-infested plants were more heavily rusted than the non-smutted.

The present investigation provides another example of the intensifying effect of one of the smuts on other diseases. It is, however, different from the cases just cited, because under field conditions, seedling blight occurs before the symptoms of flag smut would ordinarily appear or even be suspected. Jarrett (13) states that certain wheat varieties "may show infection in one or two tillers only, or be quite healthy, while microscopic examination will reveal the fungal hyphae in the base of the plant. Record of this type of infection is lost in the usual infection counts, and the actual effect of the disease can only be estimated by an analysis of yield." According to Noble (17) and Verwoerd (21), flag smut does not appear earlier than the fourth or fifth leaf stage. Jarrett (13) states that flag smut symptoms usually appear very much later in plants grown in the field than under artificial conditions.

In the mixed inoculum plot in the field, not only did a higher percentage of seedling blight occur than in plots with either organism alone, but a difference was observed between the amount of blighting of the varieties susceptible or resistant to flag smut, being greater among the former than the latter. The percentages in Table 3 show this clearly.

That the two organisms may occur together on the grain or in the soil, or that smutted grain may be planted in soil containing the foot rot organisms, obviously happens in our wheat-growing areas, and according to this work, the combined attack results in seedling-blight.

It appears probable that flag smut or other smuts in combination with one or another of the foot rot organisms may, under certain environmental conditions, produce definite basal lesions on the culms of older plants, and other symptoms of foot and root rots. Sampson and Walters Davies (18) found that bunted plants were more than ordinarily susceptible to attacks by *Fusarium* spp., and were of the opinion that this was the probable reason for the cause of mortality among bunted plants. The results reported here on the combined effect of *U. tritici* and *F. culmorum* are similar to those just cited.

Further study of the reaction of cereals to infection by the smuts and root-rotting organisms is in progress.

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Carbon Tetrachloride.

A Note on its Toxicity for Sheep.

*By D. T. Oxer, B.V.Sc.**

The observations described in the brief note that follows were made by Mr. Oxer when he was an officer of the Tasmanian Department of Agriculture and when he was working on a programme of research being carried out by the Council under the Australian Pastoral Research Trust-Empire Marketing Board scheme (see this *Journal*, 4: 133, 1931).—Ed.

1. Introduction.

The toxicity of carbon tetrachloride for sheep has from time to time been given some prominence in Australia. Ross and McKay (1), in advocating its use for the elimination of the liver fluke, have reviewed some phases of the question, while, more recently, Rose (2) has surveyed mortality following treatment of flocks in New South Wales.

Although they have been previously recorded (3), we here wish to emphasize toxic symptoms which occurred in an experimental flock of sheep following routine treatment with the drug. These sheep were being used in certain experimental feeding trials carried out in Tasmania in relation to pregnancy paralysis of ewes.

2. Toxic Symptoms in the Experimental Flock.

The flock consisted of 118 full-mouth and six-tooth Romney-Southdown ewes, which had been mated to Southdown rams. The ewes were in very good condition, and had been selected as being of a type susceptible to the disease under investigation. They were divided into four groups, each being maintained on such a small area (less than one acre) that hand-feeding was their sole means of sustenance. The rations consisted of a mixture of best oaten and lucerne chaff, with the addition of a small amount of oats and bran in the case of three of the groups. Salt was available as a lick, while one-half ounce of bone flour per sheep per day was mixed in the rations for a period of three months prior to the fourth drenching.

It was thought that such a concentration of sheep on a small area would ultimately lead to a heavy parasitic infestation, particularly as the same ground had been heavily stocked with sheep a short time previously. To keep the parasitic infestation as low as possible, it was considered advisable to commence routine treatment with carbon tetrachloride. This was accordingly carried out, the dose used being 2 c.c. of the drug in 3 c.c. liquid paraffin. The best quality procurable was obtained and was mixed in the laboratory prior to treatment.

The use of the drug was followed by toxic symptoms, which would have been classified as mild had not the exact amount of feed consumed per day been known, for, apart from loss of appetite, the only symptoms

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shown were somnolence and lethargy. No noticeable gastro-intestinal disturbances were present. The appearance of toxic symptoms was rather unexpected, but those shown after the first two treatments did not appear sufficiently severe to warrant discontinuance of treatment. The effect of the third, however, was serious, and as a fourth showed that toxic symptoms were equally severe as those previously seen, drenching was discontinued.

In Table I., figures are given showing that the appetite was greatly depressed, that this depression appeared at varying periods after treatment, and that the period of inappetance tended to increase with each successive administration of the drug.

TABLE I.

—	First Drenching.	Second Drenching.	Third Drenching.	Fourth Drenching.
Average daily amount (over one week) eaten by all groups before drenching	240 lb.	400 lb.	400 lb.	441 lb.
Average daily amount eaten during period of inappetance	150 lb.	276 lb.	266 lb.	241 lb.
Appearance of symptoms after drenching	2nd day	Same day	Same day	1st day
Return to normal	4th day	6th day	11th day	9th day

Appetite was slightly depressed at the beginning of each "toxic period," and then gradually returned to normal. The greatest reduction in the amount of food eaten occurred on the first day after the fourth treatment, when only 30 lb. of food were eaten by all groups. The loss of, and return to, appetite following the third and fourth drenchings are illustrated graphically in Fig. 1.

Drowsiness was well marked at the onset, but had the sheep been under field conditions, it would have taken close observation to detect anything unusually wrong, apart from the symptoms in the initial stages of each period.

3. The Effect of Carbon Tetrachloride on the Liver.

In dogs, toxic symptoms following the usual latent period of some hours, are constantly accompanied by central necrosis of the liver lobules. Gardner et alii (4) state that this necrosis, which may be caused by doses as low as 0.176 c.c. per kilo of body weight, may be observed as early as 8 hours, and that the maximum damage occurs in 48 hours after treatment. According to Davis (5), even extremely

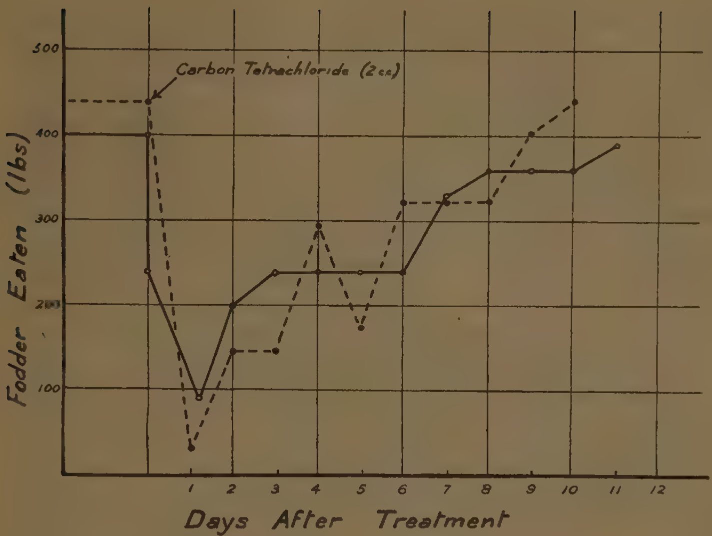

FIG. 1.—Graphs illustrating loss of appetite in sheep after drenching with 2 c.c. of carbon tetrachloride. The full line illustrates the effect of the 3rd drenching, and the dotted line the effect of the 4th drenching of the same animals some 4 weeks later.

small doses will be followed by liver injury as judged by fatty degeneration. Lamson and Wing (6) consider that a threshold dose of from 0.5 c.c. to 1 c.c. per kilo is necessary before liver necrosis can occur. Minot (7, 8) and Cutler (9, 10) consider that an excess of guanidine in the blood, following liver necrosis, is responsible for the symptoms shown, this being supported by the fact that the injection of guanidine hydrochloride gives rise to symptoms identical with those caused by carbon tetrachloride, and that both are relieved by calcium therapy. Factors other than liver necrosis are necessary, however, for the production of clinical symptoms, as it has been shown (6) that, notwithstanding a tremendous amount of liver necrosis following large and frequently repeated doses, dogs may not only remain normal to all appearances, but even gain in weight.

With the exception of Rose's statement that livers of sheep which died from the effects of carbon tetrachloride showed no evidence of liver necrosis on microscopic examination, there are no references, in the literature available, to the pathology of the liver in sheep and cattle which have succumbed to this drug. Seddon (11) has informed us that in no case has he found liver necrosis in sheep which have died following administration of therapeutic doses up to 2.5 c.c. Ross (12) also has found no evidence of necrosis following therapeutic doses, although in some sheep treated at regular intervals he has found indications of fatty degeneration.

With a view to determining whether a large, as compared with a therapeutic dose, is capable of causing liver lesions, the following experiment was carried out.

Two sheep were used. Both were in a fat and well-nourished condition. For the previous four months they had been fed on oaten chaff, in addition to which they had had access to small paddocks in which grass picking was available.

Sheep No. 1, weighing 115 lb., was drenched on 31st July, 1933, with 2 c.c. carbon tetrachloride in 3 c.c. liquid paraffin. On the first day after treatment, there was noticeable loss of appetite with a tendency to remain aloof from the others. The sheep was slaughtered 52 hours after treatment. No abnormality was revealed on post-mortem examination. Microscopically, the liver appeared normal, but hyaline degeneration of some of the convoluted tubules of the kidney was present.

Sheep No. 2, weighing 105 lb., was drenched on the same date with 15 c.c. carbon tetrachloride in the same proportion of oil. Similar, though more marked, symptoms occurred as in the case of Sheep No. 1. Post-mortem examination revealed a marked lobular necrosis of the liver, involving, on a rough estimate, about 50 per cent. of liver tissue. The internal fat showed a slight, but distinctly yellowish, discolouration. No other abnormalities were observed. On microscopical examination of the liver, necrosis of cells in the central portion of the lobules, accompanied by intense congestion, was seen. The kidney was normal.

Although a small experiment, this indicates that a therapeutic dose of carbon tetrachloride causes no liver necrosis, although a large dose may do so to a very marked degree. It also indicates that there may be a different mechanism of intoxication in sheep to that occurring in dogs, and is confirmatory evidence that liver necrosis is not essential for the production of toxic symptoms.

4. Discussion.

Considerable experience has now been gathered regarding the toxicity of carbon tetrachloride for those animals in which its therapeutic use has been most practised, and on reviewing the literature on the subject, one is at once struck by the similarity in different species of (*a*) the wide extremes in tolerance, (*b*) symptoms, and (*c*) post-mortem lesions—with the exception of sheep which, although agreeing in other respects, do not show liver necrosis following the usual medicinal doses.

With dogs, in which the greatest amount of experimental work has been carried out, it has been shown that certain conditions predispose to toxic symptoms, while other conditions seem to have the reverse effect. Briefly summarizing these, it has been shown that starvation, the addition of alcohol to the food, and diets rich in protein or fat or deficient in calcium, are factors for toxicity, while normal mixed diets or those rich in calcium or carbohydrate are factors for safety. Acidity or alkalinity of the food may possibly be factors for toxicity or safety. Magnesium sulphate may increase the safety of the drug.

Owing to the similarity of reaction to the drug in various animals, one would expect that the results of experimental work in dogs would also apply, in principle, to animals of other species. This view is supported by the experiments of Hindmarsh (13), in which he has

shown that cattle, after being rendered deficient in calcium and phosphorus, may be made susceptible to, or tolerant of, the drug by withdrawing or feeding bone meal. Field evidence indicates that the same condition holds for sheep, although we are aware of very "deficient" flocks which show no adverse effects following even regular routine treatment. Other factors suspected of rendering sheep more susceptible are the feeding of concentrates, depasturing on certain crops, or an excessively fat condition. In addition, Rose has suggested that cold, bleak weather may be a predisposing cause. The fact that sheep do not show liver necrosis may be explained by the difference in metabolism in the herbivorous, as compared with the carnivorous, animal.

In the experimental flock described, it is noteworthy that the after effects of the third and fourth drenchings were more severe than those of the first and second. This may have been due, partly, to the effect of repeated treatments, but more probably to an increasing time on a ration which rendered the animals susceptible. The component part of the ration responsible for this susceptibility may have been either the oat grain or the lucerne chaff or both. Calcium deficiency may definitely be eliminated, as, apart from the ration being of itself fairly plentifully supplied with this element, bone flour, which was mixed through the ration, failed to decrease the toxic symptoms shown. From experience with this and other flocks, adverse weather conditions are not considered to have been of importance in rendering this flock susceptible.

Although the actual cause of symptoms in these sheep is uncertain, it is considered important that such a marked effect on appetite can be caused, and yet only mild symptoms of toxaemia result. Rose has shown that in the field a dose of 1 c.c. of carbon tetrachloride may be followed by severe mortality, and in some cases by loss of wool in the survivors. It is more than probable, therefore, that in quite a large percentage of flocks regularly treated with 2 c.c. a marked loss of appetite may be shown, extending over possibly quite long periods, although only accompanied by mild symptoms and unobserved under field conditions.

It is conceivable that the loss of weight and the likelihood of faults in the wool following such severe checks would counterbalance the beneficial effect of the drug, especially when, as is often the case, it is being used for the control of a not very heavy worm infestation. Ross and Graham (14, 15) have shown that, under the conditions of their experimental trials, no such ill effects, as judged by loss of body weight and examination of the wool did occur, and Marston (16), in the experimental flocks under his control, and in which regular weighings are practised, has no reason to suspect that the routine drenching with the drug has caused any ill effects, but it must be remembered that these experimental flocks represent a very small proportion of the total flocks treated throughout Australia, and do not, therefore, necessarily prove that such adverse effects do not occur. On the contrary, the experience recorded above proves that they may occur.

In carbon tetrachloride, the veterinarian has in his hands a very efficient agent, but it must be borne in mind that it is a therapeutic and not a preventive agent. There are, however, contra-indications

for its use, of which one is definitely calcium deficiency. Regarding the others, there is at present some confusion and, although it can be said that, as in the dog, they rest mainly on the effect of diet or constituents of diet on metabolism, they require to be more accurately defined before the drug can be regarded as safe for wide and general use.

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Fruit Bud Studies.—II. The Sultana: Differentiation and Development of the Fruit Buds.

By C. Barnard, M.Sc., and J. E. Thomas, B.Sc., B.Agr.Sc., B.V.Sc.†*

Summary.

1. The results of a study of the differentiation and development of the fruit buds of the sultana vine at Merbein, Victoria, during five consecutive seasons, are reported in this article.

2. It is shown that:—

(i) Tendril and inflorescence primordia differentiate from morphologically similar anlagen.

(ii) The development of anlagen as inflorescence primordia or as tendril primordia is correlated with the time of the season during which the growth of the anlagen occurs.

(iii) Differentiation of anlagen into inflorescence primordia takes place during late spring, summer, and autumn; whilst anlagen which are initiated after the winter rest become tendril primordia. Differentiation of an anlage into a primordium is defined as the acquisition of the mode of growth characteristic of the organ developed therefrom.

(iv) Anlagen which are initiated in late summer or autumn and have not developed sufficiently before winter to acquire a definite habit of growth become tendril primordia during the following spring, whilst anlagen which had just begun to acquire the inflorescence mode of growth in autumn, develop into "transition" forms when growth recommences.

(v) Differentiation of the flowers of the inflorescence occurs as the buds are opening in spring and prohibits further ramification of the inflorescence.

3. It is concluded that:—

(i) The number of anlagen with differentiate as inflorescence primordia is determined by the rate of their development during summer and autumn. The rate of development of the anlagen during this period is therefore an important factor in determining the yield of the following season.

(ii) The potential size of the inflorescences is determined by the amount of growth made by the primordia prior to bud burst in spring.

(iii) Conditions which induce an early cessation of elongation growth in the shoots, and are favorable to the rapid accumulation of starch in the wood, appear to be conducive to the differentiation of anlagen as inflorescence primordia and their rapid development as such.

1. Introduction.

In the first paper of this series[1] it was concluded, *inter alia,* that—

(*a*) yield in the sultana is correlated with the number of fruit buds formed in the previous year.

(*b*) the number of fruit buds formed is low towards the base of the cane, increases progressively outwards, and falls off towards the distal end in all except very short canes.

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(1) This *Journal,* **5**: 47–52, 1932.

(*c*) the seasonal variation in the proportion of fruit buds produced is considerable, and is largely dependent upon the number of fruit buds formed on the distal half of the cane, and

(*d*) the appearance or initiation of the primordia (then designated "differentiation of the primordia") could be determined by microscopic examination, and, in the season of 1928-29, occurred in the basal buds about the 12th of November.

The present paper records the times of initiation of the primordia for a further four seasons, and describes their development from the time of differentiation to flower production in the following spring.

The additional data secured during these studies, however, render it necessary to modify the terminology used in the first article of the series[1]. The primordia, at the time of their initiation, are not necessarily potential inflorescences, and in fact they may develop into any one of the following three structures:—(i) inflorescences, (ii) transition forms between inflorescence and tendril, and (iii) tendrils.

The actual differentiation of the primordia as inflorescence primordia occurs relatively slowly and progressively as the primordia acquire the mode of growth characteristic of the inflorescence. The term anlagen is therefore applied hereafter by us to primordia which have not yet differentiated into one of the above three structures[2].

The facts which have led to this conception and the importance of making this distinction are explained in Section 2 (A). Suffice to say that this interpretation helps to explain the distribution (*vide* (*b*) above) of fruitful buds on the cane, and also throws considerable light on the causes of the variation in the proportion of fruit buds formed from year to year. At the same time, the importance of the period during which the first anlagen are formed is diminished by the fact that the differentiation of the anlagen into inflorescence primordia takes place progressively and may continue throughout the season. The fact remains, however, that the anlagen produced early in the season do, almost without exception, differentiate into inflorescence primordia. It has to be determined, therefore, whether the time of the initiation of the first anlagen has any influence upon their differentiation into inflorescence primordia and subsequent development.

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(1) This *Journal*, 5: 47–52, 1932.

(2) The term primordium is usually applied to a developing organ or part of an organ during its early stages of growth. The term, qualified by a substantive such as leaf, flower, or sepal, is used synonymously with the term rudiment, initial, or anlage. A primordium, so qualified, designates the organ from the time of its origin as a localized meristematic growth until the time when all the parts of the organ have been differentiated. A rudimentary flower, which has differentiated calyx, corolla, and androecium initials, but in which the gynaecium initial has not arisen, is still termed a flower primordium.

In the case of a rudiment which may develop into one of several types of structures, there is no single term which can be applied to the rudiment prior to the development of the mode of growth characteristic of one of the structures. A rudiment, for example, which may develop as a foliage leaf, a scale, or a bract, is spoken of as a cataphyllary primordium. As soon as characteristics of leaf scale or bract develop, it becomes a leaf, scale, or bract primordium.

It would appear desirable, however, to modify the terminology so that the same term is not applied to both the undifferentiated and the differentiated primordium. In view of the fact that the terms "primordium" and "rudiment" are the most commonly used ("*initial*" being generally regarded as indicating histological origin), it has been deemed preferable to select the term "*anlage*" to designate the undifferentiated primordium. The term "primordium" (or "rudiment") is, in this paper, restricted to describe developing structures which exhibit the characteristic mode of growth of the resultant mature structure or organ.

2. Investigational Work.

A. Initiation of Anlagen during Five Seasons.

The results obtained during the past five seasons are summarized in Table I. The data show that the time of initiation of the first anlage varied slightly in different seasons, taking place earliest in 1931 and 1930, and latest in 1932 and 1928. It has not been possible, however, to correlate the time of anlagen initiation with the tendency to form a high proportion of inflorescence primordia. Counts, made in the spring, of the number of fruitful shoots, together with the yields at the subsequent harvests, are also shown in the Table. Under normal conditions bud counts and subsequent yields are fairly closely correlated; this fact is evident from the figures of the experimental plot. Variations in the times of initiation in different seasons are not large enough to warrant any comparison with subsequent bud development or resultant yields. However, if any conclusion is to be drawn from the data, it is to the effect that the number of fruit buds formed in any season is not related to the time of initiation of the first anlage in the buds.

TABLE I.—PERIOD OF INITIATION OF ANLAGEN IN SULTANA BUDS DURING FIVE CONSECUTIVE SEASONS, TOGETHER WITH BUD COUNTS DURING THE SPRING AND YIELDS AT HARVEST FROM ONE EXPERIMENTAL FIELD.

Time of Initiation of First Anlage.	1928.	1929.	1930.	1931.	1932.
Buds 1–4 of shoot (Base)	First fortnight in Nov.	End of Oct. and first week in Nov.	End of Oct.	End of Oct.	First week in Nov.
Buds 5–10 of shoot (Middle)	Third and fourth weeks in Nov.	Mid-Nov. in rapid succession	Mid-Nov. in rapid succession	First fortnight in Nov.	Mid-Nov. and third week in Nov.
Buds 11–16 of shoot (End)	Second week in Dec.	End of Nov. and first week in Dec.	Mid-Nov. to third week in Nov.	Mid-Nov. to third week in Nov. in rapid succession	First week in Dec.
	1929.	1930.	1931.	1932.	
Percentage bud count in following spring. Fruitful shoots of total buds	42%	23%	35%	37%	
	1930.	1931.	1932.	1933.	
Yields at subsequent harvest. Dried weight, tons per acre	1·85	0·47	1·38	1·80	

In most other fruits, the fruit buds are either produced on spurs or on comparatively short shoots, and the development of the buds on different parts of the shoot or tree takes place more or less synchronously. Under such circumstances, it is possible without much difficulty to

examine sufficient material to warrant a generalized statement applicable to the season. In the vine, on the other hand, the fruit buds are produced on long shoots, and they develop in acropetal succession. It is necessary, therefore, to determine the time of initiation in each bud from the base to the apex of the shoot, and to replicate these observations, at each examination during the season.

In view of the difficulties involved in determining the time of initiation for the season, and also in consideration of the unpromising nature of the results obtained over a period of five seasons, the attack on the problem along these lines has been discontinued.

B. Fruit Bud Development During Summer and Autumn.

The structure and organization of the buds as well as the differentiation of the anlagen into inflorescence primordia have been described in the first article of this series. The following account continues the description of the development of the buds during summer and autumn.

By the middle of November, the eighth leaf rudiment has arisen in the majority of buds. The first anlage to arise is usually situated opposite this leaf, and, if developed, differentiates later into an inflorescence primordium. Occasionally, an anlage is developed at the 7th embryonic node, but only rarely at a lower one. On the other hand, if the first anlage is developed opposite the ninth leaf, it may or may not develop as an inflorescence primordium.

By the beginning of December, the ninth leaf is arising in most buds, though the 10th, 11th, and 12th leaf rudiment may have been formed in the buds borne on the lower portions of the shoot. Towards the middle of December, the 10th leaf rudiment arises and very often a 2nd anlage may be observed at the ninth embryonic node.

Development generally ceases when twelve to thirteen leaf rudiments have been formed, though the time at which it occurs varies according to the position of the bud on the shoot. In the basal buds, the 12th or 13th leaf rudiment has generally been formed by the end of December, whilst the majority of buds have not progressed to this stage until about the middle of March.

The stage of development reached by a number of buds, collected from the 9th to 11th nodes during March, is shown in Table II.

The first 14 buds represented in the Table are strong and advanced, whilst the remainder are more typical of the normal fruit bud. At this time, the first formed anlage, usually opposite the 8th leaf rudiment, has developed into a comparatively large and well formed inflorescence primordium which has attained the proportions represented in Table II. The anlage opposite the 9th leaf, however, varies considerably in size, and only shows the characteristics of an inflorescence primordium in advanced buds. In this connexion, it will be observed from Table II. that the anlagen, by reason of their dimensions, fall into two fairly distinct classes. Firstly, those at the 8th and 9th embryonic nodes in the advanced buds and those at the 8th in the normal buds form a class with dimensions greater than 0.40 x 0.30 mm. The anlagen at higher embryonic nodes fall into a group having dimensions less than 0.20 mm. x 0.18 mm. It may safely be stated that the primordia included in the 1st class will develop into inflorescences, those in the 2nd class into tendrils, and those of intermediate dimensions will in all probability become transition forms between tendrils and inflorescences.

TABLE II.—THE DIMENSIONS IN MILLIMETRES AND POSITION OF ANLAGEN DEVELOPED IN 25 BUDS ON 16.3.1932.

——	7th Node.	8th Node.*	9th Node.	10th Node.	11th Node.	12th Node.	13th Node.
1 ..	..	·80 x ·68	·40 x ·32	..	·08 x ·08	·06 x ·06	..
2 ..	..	·68 x ·56	·48 x ·32	..	·08 x ·08	·06 x ·04	..
3 ..	..	·76 x ·40	·40 x ·32	..	·12 x ·10	·06 x ·06	..
4 ..	·80 x ·80	·52 x ·44	..	..	·16 x ·16	·08 x ·10	..
5 ..	..	·72 x ·68	·56 x ·52	..	·12 x ·08	·08 x ·08	..
6 ..	..	·64 x ·56	..	..	·20 x ·12	·06 x ·06	..
7 ..	..	·80 x ·76	·64 x ·56	..	·12 x ·06	..	Arising
8 ..	..	..	·60 x ·68	..	·12 x ·08	..	Arising
9 ..	..	·72 x ·72	..	·16 x ·12	..	..	Arising
10 ..	..	·70 x ·64	·40 x ·36	..	·12 x ·16	·10 x ·10	..
11 ..	..	·72 x ·72	·60 x ·48	..	..	·10 x ·08	..
12 ..	..	·64 x ·72	·32 x ·24	..	..	·12 x ·12	..
13 ..	..	·68 x ·60	..	·16 x ·16	·08 x ·08	..	Arising
14 ..	..	·80 x ·60	..	·12 x ·12	·10 x ·08	..	Arising
Percentage frequency	7	93	65	21	70	65	36
Mean area Sq. Mms.	·64 (·80 x ·80)	·44 ± ·03 (·71 x ·62)	·22 ± ·04 (·48 x ·42)	·02 (·14 x ·13)	·012 ± ·002 (·10 x ·11)	·007 ± ·001 (·08 x ·08)	.. ..
15 ..	..	·72 x ·72	·20 x ·16	..	·08 x ·06	Arising	..
16 ..	..	·48 x ·36	·16 x ·16	..	·08 x ·04	Arising	..
17 ..	..	·40 x ·32	·16 x ·16	..	(·04 x ·04) Arising	..	..
18 ..	..	·48 x ·40	·12 x ·08	..	..	Arising	..
19 ..	..	·52 x ·44	..	·12 x ·08	·08 x ·06	..	..
20 ..	..	·52 x ·44	·28 x ·24	..	·08 x ·08	..	..
21 ..	..	..	·16 x ·14	·12 x ·12	..	..	..
22 ..	..	·56 x ·56	·12 x ·08	..	·08 x ·06	..	..
23 ..	..	..	·36 x ·36	·12 x ·14	..	Arising	..
24 ..	..	·52 x ·44	·12 x ·14	..	·06 x ·06	..	..
25 ..	..	...	..	..	·20 x ·16	·06 x ·06	..
Percentage frequency	0	73	82	27	73	45	0
Mean area Sq. Mms.	0 ..	·25 ± ·05 (·53 x ·46)	·36 ± ·01 (·20 x ·17)	·013 (·12 x ·11)	·007 ± ·001 (·09 x ·07)	.. ..	.. ..

* Measurements show length and breadth of primordia and are representative of measurements made in a collection of 100 buds. Measurements made by means of a micrometer and dissecting microscope.

The characteristic mode of growth in the inflorescence primordia may be described as a tendency to originate numerous growing apices. Large well formed inflorescence primordia have the appearance of a closely packed bunch of grapes (Figs. 1, 2, 3, Plate 4)*, each "grape" representing a growing point. The primordium is a very complex branch system in which the branches elongate slowly but continue to divide rapidly.

* For Plates see facing page 308.

At the time of its origin, the anlage is simply a pad of meristematic tissue. As it enlarges, it develops into a bulbous protuberance which splits off a second growing point towards its base. This second apex represents the initial of the lowest branch of the system, and anlagen up to, and in this condition fall into the second of the two classes referred to above. Earlier formed anlagen rapidly develop the characteristic mode of growth of the inflorescence, and the primordium enlarges by the repeated formation of fresh apices. The mode of growth followed by anlagen which differentiate as tendril primordia is essentially different and will be described in the following section.

It will be noted that the disposition of anlagen on the axis of the bud usually follows a definite plan. They arise opposite the 8th and 9th leaves, 11th and 12th, and at all except every third successive embryonic node, and may be represented as follows:—AA—AA—AA—etc.

Very often, an anlage is not developed at the 11th or 12th embryonic node and less frequently no anlage is present at the 9th. It is only occasionally, as pointed out previously, that an anlage arises at an embryonic node lower than the 8th.

Development gradually ceases in autumn, and the buds pass through a dormant period during winter in very much the same condition as they have reached by the end of March. The small amount of growth which takes place between mid-March and dormancy does not materially affect the relative development of the anlagen.

C. Fruit Bud Development Prior to Bud-Burst in Spring.

The appearance of the inflorescence primordium during the first half of August is similar to that represented in Fig. 2, Plate 4. Growth recommences about the middle of August, and is slow at first but increases progressively until the buds open about the first week in September. The inflorescence primordia increase considerably in size (Fig. 3, Pl. 4), and the axis of the bud grows forward forming new leaf rudiments and anlagen (Fig. 4, Pl. 4). Anlagen initiated during this period and during subsequent growth develop into tendril primordia (at "t", Fig. 4, Pl. 4). The mode of growth of the tendril primordia is essentially different from that of the inflorescence primordia. In the former, only a few growing points are formed, and these elongate rapidly to become branches. The tendrils, as well as the tendril primordium, may be described as branch systems in which the branches elongate rapidly but only divide occasionally.

The anlagen which had reached a stage comparable to that shown at "a" in Figures 4 and 6, Plate 4, or were slightly more advanced, prior to the recommencement of growth during August, develop as tendrils also. These anlagen are comparable to those represented at the 10th and succeeding nodes in the advanced buds of Table II. (at "a" in Fig. 1, Pl. 4) and the majority of those at the 9th node in the normal buds (i.e, the group of anlagen less than 0.20 x 0.18 mm. in size during March).

Finally, the anlagen which were less than 0.40 x 0.30 mm. in size and greater than 0.20 x 0.18 mm. during March generally develop in spring to produce structures which are transition forms between tendrils and inflorescences. These transition forms are not of very frequent occurrence as may be determined by examination during early spring

subsequent to bud burst, but all gradations from perfect inflorescences to perfect tendrils may be found. In this connexion, it is interesting to note that in the majority of inflorescences, however, one branch is in the form of a tendril.

Primordia of typical transition forms are illustrated at "tr", Figs. 5 and 6. The primordium in Fig. 5 will probably develop into a tendril with perhaps a few flowers on some branches, whilst that represented in Fig. 6 may become more like a true inflorescence and produce a number of flowers.

It will be apparent, therefore, that the anlagen at the time of their origin are potentially similar, and that the inflorescence and tendril are homologous structures. Under the conditions controlling growth during late spring, summer, and autumn, anlagen develop into inflorescence primordia. Conditions under which growth recommences in spring, however, are totally different and are associated with the rise of the sap. Under these conditions, anlagen develop as tendril primordia.

In view therefore of the facts that—

(*a*) well developed inflorescence primordia continue to develop in spring in the same manner as in the previous season, and

(*b*) medium sized anlagen, which have begun to develop into inflorescence primordia, change their mode of growth when encountering spring conditions and develop into transition types instead,

it seems reasonable to assume that—

(i) the mode of growth characteristic of the inflorescence is acquired by anlagen during their development in late spring, summer, and autumn, and when acquired this growth mode persists in spring, notwithstanding the changed conditions.

(ii) anlagen, which have formed late in the season, and which, therefore, have not acquired a characteristic mode of growth, develop when new growth conditions are encountered in the spring entirely according to those conditions.

(iii) anlagen, which have begun to grow in the way of inflorescence primordia, but which have not differentiated sufficiently to acquire this characteristic, are so affected by the new conditions in spring that their mode of growth is altered.

D. Flower Differentiation and Development.

The inflorescence primordia, as noted in the previous section, increase considerably in size during the period following the rise of the sap in mid-August. The differentiation of the flowers, however, does not occur until the time when the buds are on the point of bursting.

The first indication of flower initiation is the division of each growing apex into three more or less equal parts. In Fig. 1, Plate 5, a portion of a large inflorescence primordium, prior to initiation, is depicted, and in Fig. 2 of the same plate a smaller portion of another inflorescence

is shown, in which the division of the growing apices into three is illustrated. Each of the three growing points represents a flower primordium.

Differentiation occurs over the whole inflorescence almost simultaneously, and takes place in the basal branches only very slightly in advance of the apical branches. As soon as differentiation is accomplished, therefore, further branching of the inflorescence is prohibited. This fact is to be specially noted, as it means that the number of flowers which an inflorescence may produce is determined by the amount of growth which is made by the inflorescence prior to the differentiation of the flowers. Most of the growth and branching of the inflorescence occurs during the period from mid-August to bud-burst, but it is highly probable that the extent of the growth of an inflorescence during this period is very largely dependent upon the stage of development it had reached at the end of the previous autumn.

In Figs. 3-6, Plate 5, the development of the flower is illustrated. In Fig. 3, the sepals have been formed, whilst in Fig. 4 a more advanced stage is depicted in which the stamens are arising.

It is an interesting fact to note that the flowers in different inflorescences and in different parts of the same inflorescence all reach the same stage of development at the one time. This feature was particularly noticeable in material collected on the 23rd September, 1930 (Fig. 4). Apical and basal branches of many inflorescences were examined, and, in each case, the flowers were at approximately the same stage in their development.

Shortly after the origin of the stamens, and prior to the initiation of the carpels, the petals curve inwards and downwards, and fuse together along their ventral surfaces. The outer cells of the incurved portion of the petals are irregularly and loosely disposed. Growth of the petals forces their surfaces together, the loosely disposed surface cells becoming interlocked. Further division of these cells completely establishes the connexion. The fusion of the petals results in the formation of the corolla lid or operculum, which separates circumscissilly when the flower opens. In Fig. 5, Plate 5, the fusion of the petals and the origin of the carpels are illustrated. Further growth results in the development of the stamens and carpels and origin of the ovules.

In 1930, pollen was formed on the 6th-7th of October by the tetrad division of the spore mother cells, and the megaspore was distinguishable in the ovule by the 13th. Further development was not followed in detail, but a large and apparently normal embryo sac had developed by the 24th October, just prior to the opening of the flower.

In 1929, development was later than in 1930 throughout the season.

The fact that the differentiation of the flowers of the inflorescence does not take place until the buds are on the point of opening in spring has not so far been generally recognized. Goff 1901[1] was of the opinion that flowers are unquestionably formed during the season previous to their expansion (in the grape). Chandler[2] misquotes Bioletti[3] as stating that the flower parts of the grape, *Vitis vinifera*, are formed during the season before the flowers open, and this opinion

(1) "Investigations of Flower Buds." Agric. Expt. Station, Wisconsin, Ann. Rept., 1901, p. 316.
(2) "Fruit Growing." (Houghton and Mifflin, U.S.A.), 1925, p. 51.
(3) "Vine Pruning in California." Univ. Calif. Agric. Expt. Station, Bull. No. 241, Pt. 1, 1914.

is usually quoted in text books and manuals. Winkler(1), however, recognized the fact that parts of the flower were formed after the buds had opened.(2)

3. Relation to Yield.

In view of the fact that two important factors controlling yield are the number and size of the inflorescences, it becomes evident from the foregoing that more attention should be directed to the rate of development of the anlagen during summer and autumn. The destiny of the anlagen is determined by their development during this period. The number of anlagen which differentiate as inflorescence primordia is dependent upon the rate of growth of the anlagen prior to winter dormancy, and the ultimate sizes of the inflorescences are largely controlled by the amount of growth made during the same period.

A comparison of the conditions prevailing during the periods when the anlagen form inflorescence primordia and when they differentiate as tendril primordia indicates the nature of the conditions which are inducive to inflorescence differentiation.

The conditions obtaining during late spring, summer, and autumn are characterized by the gradual slowing and final cessation of vegetative growth translocation of sugars to the maturing grape, followed by the conversion of sugar products, and the storage of starch. The conditions obtaining during early spring are characterized, on the other hand, by the re-conversion of starches to sugars, commencement of vegetative growth concurrent with the rise of the sap, followed by a progressive increase in the rate of vegetative growth. A comparison of these sets of conditions suggests that the early cessation of growth in summer and the rapid accumulation of starch may be conducive to the differentiation of anlagen as inflorescence primordia.

The following instance lends strong support to this contention. In one experimental field, owing to abnormal weather conditions, an early loss of crop occurred in December, 1931. In the succeeding year, however, the bud fertility (fruitful shoots/total buds) of these vines was 60 per cent., in marked contrast to the values of from 37-40 per cent. attained in other fields where the 1931-32 crop had been harvested. It might be inferred that, consequent on the premature loss of crop, there was an early accumulation of starch within the annual wood, and this in turn tended towards the differentiation of a high proportion of anlagen as inflorescence primordia.

Experimental work, based on the conclusions drawn from the observations communicated in this article, is at present in progress. In one series of experiments, an attempt is being made to influence the development of the anlagen and primordia during summer and autumn by cultural means. The object of a second series is to obtain more information in respect to the factors influencing anlagen differentiation and

(1) "Pruning and Thinning Experiments with Grapes." Univ. Cal. Agric. Expt. Station, Bull 519, 1931, p. 47.

(2) Subsequent to the presentation of this article for publication, a paper entitled "Flower Bud Formation in the Concord Grape," by J. C. Snyder, has appeared in the *Botanical Gazette*, Vol. 94, No. 4, p. 771. Apart from minor differences, some of which are evidently of a varietal character, such as the number of inflorescences per shoot, this account of the development of the inflorescence and flower of the vine agrees with our observations. The coalescence of the calyx into a cap has not been noted by us in the Sultana, however. Snyder does not deal with the relationships between inflorescence and tendril primordia upon which the significance of our communication depends.

inflorescence primordia development. The process of initiation and differentiation on a large number of shoots is being studied each season. Growth records and measurements are carried out on these shoots, and an attempt will be made to correlate characters of vegetative growth of the shoot with the process of anlagen formation, and the tendency to differentiate a large proportion of these as inflorescence primordia.

4. Acknowledgments.

The writers wish to express their appreciation of the helpful advice and criticism tendered to them during the course of these studies by Dr. B. T. Dickson, Chief of the Division of Plant Industry, C.S.I.R.

The Effect on the Body-Weight and Wool Production of Merino Sheep of adding Sulphur to the Diet.*

By A. W. Peirce, B.Sc.†

1. Introduction.

Steyn (1931 and 1932) reported some experiments in which increases in weight and wool production of sheep followed feeding 5 to 30 gm. of sulphur to them weekly. The sheep experimented with were mostly full-mouthed, but they increased 40 and 50 per cent. in weight during the first twelve months, and a further 20 to 30 per cent. in the next twelve months, so presumably they were in poor condition at the beginning. Steyn does not mention the diet given or consumed.

Evidence at present available does not suggest that the sulphur-containing amino-acid, cystine, can be synthesised by the animal organism from simpler compounds of sulphur. Westerman and Rose (1927) showed that thioglycollic acid and dithio-dipropionic acid could not be utilized for cystine production by the rat. Experimenting with the same animal, Lewis and Lewis (1926) and Rose and Huddlestun (1926) (and Beard (1925-6) with mice), found that taurine, a derivative of cystine, does not supplement a cystine-deficient diet either for maintenance or growth. Daniels and Rich (1918) found that inorganic sulphates could not supply the cystine deficiency of a ration nor spare the maintenance demand for cystine in rats, and Geiling (1917) that flowers of sulphur did not prevent the decline in weight of mice on a diet which could be rendered satisfactory by the addition of cystine. Muldoon, Shiple, and Sherwin (1924) showed that in dogs fed with a carbohydrate diet insufficient cystine was available to detoxicate brombenzene. Sulphur supplied as free sulphur, sulphate, or sulphide was unable to bring about detoxication, whereas cystine could do so. Even d-cystine cannot be substituted, in the rat, for the naturally occurring l-cystine, according to the recent experiments of du Vigneaud, Dorfmann, and Loring (1932). So far, the only substance discovered which

* Submitted for publication 7th April, 1933.
† An officer of the Division of Animal Nutrition, C.S.I.R., located at the Division's head-quarters, University of Adelaide.

seems to be able partially or wholly to replace cystine in a diet is the amino-acid methionine. The recent observations of Jackson and Block (1932) suggest this conclusion.

The primary object of Steyn's experiment appears to have been to ascertain the amount of sulphur which could be added to the diet of a sheep without poisoning it. He points out (p. 491) the defects of his experiment, and that before coming to any definite conclusions as to the effects of sulphur on the weight and wool production of sheep it would be advisable to conduct experiments on groups of sheep of more homogeneous compositions than those he employed.

However, as the average increment of weight in the sheep observed by Steyn improved with the increase in the amount of sulphur given, and the wool production in those receiving sulphur was 10 to 40 per cent. greater, it was decided to undertake an experiment to see whether the addition of flowers of sulphur (brimstone and treacle) to a diet, the protein of which was largely furnished by casein which has a low cystine content, would lead to the growth of more wool. On this diet, experience had shown that wool production was below normal.

2. Arrangement of Experiment.

As the experiment was concerned mainly with wool production, mature sheep were selected in order to eliminate complications caused by growth which would have intervened had young animals been used. Eight ewes almost five years old, and two wethers nearly four years old, were divided into two groups each containing four ewes and one wether. The difference between the mean weights of the two groups after shearing was 5 per cent., and the groups were also matched so that they were as similar as possible in wool character. The lightest and heaviest sheep weighed 32 and 52 kg. respectively. The animals were in fair condition only at the beginning of the experiment, as they had been grazing on natural pasture which is poor in Adelaide in autumn.

The basal diet consisted of a mixture of 420 gm. of chaffed wheaten straw, and 240 gm. of chaffed wheaten hay, together with 100 gm. of molasses, 50 gm. of casein, and 15 gm. of linseed oil per day. This ration, which contained approximately 62 gm. of digestible crude protein, 430 gm. total digestible nutrients, 1,500 calories metabolizable energy, and 240 gm. of starch equivalent, proved to be a slightly super-maintenance ration for the ewes, although not for the wethers, which were about 30 per cent. heavier; it was decided, however, to feed the same ration to both ewes and wethers.

One group of sheep received 2 gm. of flowers of sulphur daily in addition to the basal diet.

The sheep were brought into pens from pasture in the middle of April, 1932, and were first fed hay chaff with which was mixed a gradually increasing proportion of straw, until, by the end of a fortnight, they were receiving the experimental ration. This was given in two portions. The first, in the morning, consisted of some straw and hay mixed with the daily ration of molasses. To this was added casein in the case of the control animals, and the casein-sulphur mixture in the case of the sulphur-fed sheep. Within a short time, this feed was entirely consumed, thus ensuring that the sulphur was taken. In the afternoon, the remainder of the straw and hay was fed, together with

the linseed oil which was poured over the roughage. With the exception of one of the controls which invariably left a small amount, this portion of the ration was also consumed. After about five months, all the sheep, but particularly the sulphur group, began to leave some of the second meal, so that it was decided to feed hay chaff instead of hay and straw at this feed. This resulted in change in the total daily roughage to 360 gm. of hay and 315 gm. of straw. Following this, the animals began to eat rather better, although some of the sulphur group still left a portion of the second feed of the day uneaten.

Shearing took place at the beginning of the experiment, and the animals were again shorn seven months later, in the middle of December. The greasy wool cut by each sheep at this second shearing, representing the growth in 32 weeks on the experimental diet, was weighed. The wool from the individual sheep was then put through a fleece breaker of the type described by Wilson (1928). A representative sample of this "willeyed" wool was then selected and scoured by the commercial method, and the total production of clean scoured wool by each sheep calculated.

Weekly weighings of the sheep took place unless wet weather made this impossible. The experimental sheep were dipped five weeks before the end of the experiment, as the remainder of the flock was then being dipped.

3. Results.

(i) *Weight increase.*

The mean weekly weights are shown in Table I. From this it will be seen that both groups of sheep increased slowly in weight at the same rate for about the first five months, the growth curves being almost parallel during this period. Following this, the decreased consumption of roughage led to a decline in weight, which was similar for both groups. With the increase in the proportion of hay chaff in the roughage, consumption increased, as also did weight. However, increase in weight occurred to a greater extent in the case of the controls, which almost overtook the sulphur group. Over the whole period, the sulphur-fed animals increased from 40.8 to 45.3 kg., a gain of 4.5 kg., whereas the controls increased from 38.6 to 44.8 kg., an increase of 6.2 kg. The weight increases, although somewhat in favour of the controls, are not significantly different, especially as the greater part of the difference occurred in the last four weeks.

The addition of sulphur to the diet of these sheep did not cause an increase in weight greater than that of the controls.

(ii) *Wool Production.*

The weight of greasy wool and the calculated clean scoured weight cut by the individual sheep, together with the mean of the groups and the quality (count) of the individual fleeces, is given in Table II. From these figures, it is seen that there is a slight difference in weight, too small to be significant, in favour of the control group. There was also little difference between the groups as regards count. Before the experiment began, the animals were paired up as closely as possible as to wool quality, in order that the two groups should be as nearly equal as possible in this respect.

TABLE I.—MEAN WEEKLY WEIGHTS.

Week.	Sulphur.	Control.	Week.	Sulphur.	Control.
	kg.	kg.		kg.	kg.
0	39·9	38·4	17	45·0	43·7
1	41·1	38·6	18	45·6	44·1
2	41·4	38·9	19	45·3	43·3
3	40·4	38·2	20	46·5	44·0
5	40·8	39·4	22	46·5	44·3
7	42·1	40·1	23	46·3	44·7
8	43·0	41·2	24	45·9	44·4
9	43·1	41·4	25	45·5	44·1
10	44·0	42·1	26	46·0	43·9
11	43·1	41·7	27	44·5	42·2
12	44·2	42·2	28	43·8	43·2
13	43·6	42·7	29	45·0	44·6
14	44·4	42·9	30	45·0	44·4
15	44·7	43·0	31	45·8	45·5
16	44·4	44·1			

TABLE II.—THE PRODUCTION OF WOOL FLEECE.

Group.	Sheep.	Sex.	Greasy Wool (kg.).		Clean Scoured Wool (kg.).		Count.
			Experimental Period (223 days).	Calculated for Twelve Months.	Experimental Period (223 days).	Calculated for Twelve Months.	
Sulphur	$W_1 27$	♀	1·96	3·21	1·270	2·080	60
	$W_1 43$	♀	1·98	3·24	1·405	2·300	60
	$W_1 44$	♀	1·91	3·13	1·125	1·840	70
	$W_1 50$	♀	1·91	3·13	1·130	1·850	60
	$W_2 51$	♂	2·48	4·06	1·660	2·715	60–64
	Mean	..	2·05	3·35	1·32	2·16	..
Control	$W_2 31$	♂	2·51	4·11	1·630	2·670	60–64
	$W_1 2$	♀	2·07	3·39	1·485	2·430	58
	$W_1 14$	♀	2·35	3·85	1·460	2·390	60
	$W_1 34$	♀	1·95	3·19	1·175	1·925	64
	$W_1 35$	♀	2·04	3·34	1·050	1·720	64
	Mean	..	2·18	3·57	1·36	2·23	..

4. Discussion.

The experiment described by Steyn (1931 and 1932) was, as the author himself realized, unsatisfactory in some respects. There was lack of uniformity in the animals used. Their weight at the beginning of the experiment ranged from 55 to 116 lb., and, as the sheep were either full-mouthed or four-toothed animals at that time, many of them must have suffered from severe nutritional stress during their lifetime, or else were of very different breeding. The fact that the increases in weight amounted to as much as 90 per cent. during the first twelve months of the experiment shows that the food supply of the animals during the experimental period was greatly superior to that before. Unfortunately, the diets before and during the experiment are not

mentioned. It would not be just to attribute the higher average rate of increase in weight of the groups receiving sulphur to the nutritive effect of this mineral.

A similar criticism would apply to the conclusion that the sulphur increased the production of wool. The variations in the amount of greasy wool produced by sheep in the same group are so large (reaching 78 per cent. in one case, in 1930, and 107 per cent. in 1931) that it is doubtful whether there is a significant difference between the groups.

In the experiment recorded in the present paper, the sheep did not differ greatly in weight and were all mature animals. The food consumption of all the sheep, apart from the period of four weeks previously mentioned, was within 10 per cent. The largest variation between the weight of greasy wool cut by any two sheep in the same group was 30 per cent. The production of clean scoured wool, however, showed a maximum difference of 55 per cent.

The experiment shows that the weight increases were not materially different in the groups; in fact the slight difference noted was in favour of the controls. Thus, what proved to be a maintenance ration for the control sheep was also a maintenance ration for those receiving 2 gm. of sulphur per day.

The differences in wool growth were also of no significance, although they, too, were slightly in favour of the control group. Feeding of sulphur had no effect in increasing wool growth.

5. Conclusion.

The administration of 2 gm. of sulphur per day for a period of seven months did not increase the wool production of mature sheep.

While this article was in the press, one by Seddon appeared (*Aust. Vet. J.* **9**: 154, 1933) giving the results of a somewhat similar experiment. Two groups, each of 7 wethers 13 months old, even in body weight, size, and wool quality, were run together on pasture for two years. One group received 10 gms. of sulphur three times per week. The result showed that the sulphur had no material effect on body or fleece weights.

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The Microphotography of the Living Virus of Pleuro-Pneumonia of Cattle.

*By A. W. Turner, D.Sc., D.V.Sc.**

The purpose of this brief note is to introduce and explain some photographs of the causal organism or "virus" of pleuro-pneumonia of cattle.

The living organism was first observed as minute oscillating points by Nocard and Roux in 1898, when grown by them in collodion sacs in the peritoneal cavity of the rabbit.

A little time afterwards, it was cultivated *in vitro* by them in collaboration with Borrel, Salimbeni, and Dujardin-Beaumetz. In 1910, Borrel, Dujardin-Beaumetz, Jeantet, and Jouan published a paper on the morphology of the causal organism, based mostly upon stained centrifuged deposit of cultures, but they included a drawing illustrating certain forms seen by the aid of dark ground illumination. Their work was quickly challenged in Germany by Freiberger (1912), who declared the forms described by the French workers were merely precipitates, &c.

Examination by dark ground illumination does not appear to have been very successful in the hands of any of the later workers. As late as 1923, Frosch, in Germany, investigated cultures by photographing them in ultra-violet light, and found only tiny spheres and a few Y-shaped elements, and as a result considered the organism to be allied with the yeasts. Barnard, in 1925, in England, during his work with Gye on the etiology of cancer, studied cultures of pleuro-pneumonia virus by the most efficient method of investigation known to science, i.e., photography by dark ground illumination using ultra-violet light, and similarly found only tiny spherules; as a result, he suggested an extraordinary method of multiplication by a process of budding. The photographs of both Frosch and Barnard are far from satisfactory or convincing. Wroblewski (1931), who has published an excellent account of the morphology of the organism as revealed by stained preparations of cultures, could find nothing characteristic by dark ground examination of cultures. As a result of this work, he has claimed that the organism reproduces both asexually and sexually.

Interest in the morphology followed upon our introduction of a new culture medium known as "filtered V.F.-ox serum," and which gives heavy growths overnight.† Examination by ordinary dark ground illumination was consequently commenced. Enormous numbers of organisms in various stages of growth were readily found; work on its morphology and life history as revealed by a study of the living organism has been going on for about twelve months, but until recently attempts to photograph the organism have been unsuccessful, the amount of light passing through the microscope by this method of illumination being relatively small.

However, certain results have at last been obtained, and what are undoubtedly the first satisfactory photographs of the living virus of pleuro-pneumonia are presented in the accompanying photographs.‡ Our

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† This medium will form the subject of a paper to be published elsewhere. It is essentially the medium used by us in our black disease studies (C.S.I.R., Bulletin 41), containing 10 per cent. of serum and sterilized not by heat but by filtration through a Seitz E.K. disc.

‡ See Plate 6, facing page 309.

success over workers with superior equipment may be attributed partly to the culture medium, and partly to the technique used for producing the photographic records, which has been made possible by the valued assistance of Mr. A. T. Dann, M.Sc. Briefly, the photographs were taken by supporting, in contact with the ocular of the microscope, a Leica camera fitted with an Elmar F. 3.5 lens of 5 cm. focal length, at full aperture and focussed at infinity. The microscope was fitted with a 60 X apochromatic homogeneous oil immersion objective, with iris diaphragm giving a numerical aperture from 0.85 to 1.00; compensating oculars were used, the most useful being the K 15. The magnification thus given would be $60 \times 15 = 900$ if the image were projected on a screen removed the conventional 25 cms. from the ocular; but as the image was actually focussed in the camera on the sensitive film about 5 cm. from the ocular, the actual magnification was one-fifth of 900, or 180 diameters, and its luminosity was increased proportionately. The Leica camera uses cinematograph film, and makes negatives 37 mm. x 25 mm. The particular film used was Agfa Superpanchromatic, of such fine grain that the tiny negatives readily allowed the enlargements illustrated to be prepared, showing the organism at a final magnification of 1,250 diameters. Even with this film, which is probably the fastest at present known, exposures could not be reduced much under one second, and consequently some of the photographs show evidence of Brownian movement. The source of light was a Phillips 75 c.p. Pointlight with aplanatic condenser; the dark ground condenser was the Zeiss Cardiod.

A perusal of the photographs will reveal that the organism typically produces a branched mycelium that may reach a surprising size. The long filament with terminal branches in Fig. 1 is over 65μ long; the longest we have yet seen measured 155μ long. Obviously, such forms cannot be filtrable; but the organism owes this property to its extraordinary facility for fragmenting. The mycelia during observation may within a few minutes become beaded, very closely resembling streptococci, the elements of which thereupon proceed to break away as approximately spherical cocci about 0.2μ in diameter. Streptococcoid forms are produced very early during growth. Essentially similar branched mycelia can be seen in pleural "virus" of natural cases of pleuro-pneumonia, provided it is examined immediately after removal; within a few hours, only the tiny coccoid forms may be found.

Some of the photographs illustrate small spheres similar to those illustrated by Barnard and by Frosch; in cultures on serum V.F. agar, spheroidal forms predominate, many of them at closer examination revealing themselves as concavo-convex or flat discs; but it must be conceded that they are far from representing the characteristic and most striking stage of the life history; they become more common in "filtered V.F.-ox serum" as the cultures age, and are possibly another filtrable phase.

An article covering the above work in more detail will be published shortly.

Throughout the investigation, the greatest assistance has been given by my colleagues, Messrs. A. D. Campbell, B.V.Sc., and A. T. Dick, B.Sc., who have frequently drawn my attention to cultures and preparations suitable for study.

Radio Research Board: Fifth Annual Report (for the Year ended 30th June, 1933).

The Radio Research Board of the Council is constituted as follows:—Professor J. P. Madsen (University of Sydney), Chairman; Mr. H. P. Brown (Director-General, Postmaster-General's Department); Electrical-Commander F. G. Cresswell (Department of Defence); and Professor T. H. Laby (University of Melbourne). Its previous annual report was published in this *Journal* (Vol. 5, No. 4, November, 1932).—Ed.

1. General.

During the past year, the work of the Board has been continued on its former lines, the main investigations concerning (i) the reflecting layers of the ionosphere, chiefly from the point of view of fading problems, and (ii) atmospherics.

The original three year arrangement regarding the financing of the Board has now expired, but the two co-operating bodies, namely, the Postmaster-General's Department and the Council for Scientific and Industrial Research, have entered into another agreement to finance the Board's work for a further period of three years as from the 1st July, 1933. The Department will continue to meet three-quarters of the total cost of the Board's operations. This measure of stability to its investigations—so necessary if effective work is to be done—is appreciated by the Board.

Further changes in the staff of the Board have taken place. Mr. R. O. Cherry, M.Sc., resigned as from the beginning of 1933. Two new appointments have recently been made, the appointees being Dr. G. Builder, M.Sc., Ph.D., and Dr. H. C. Webster, M.Sc., Ph.D. Both of these are Australian graduates, the former from the University of Western Australia, and the latter from the Universities of Tasmania and Melbourne. For the last few years, they have been gaining post-graduate experience in Great Britain. It is expected that they will reach Australia in about September, 1933. In the first instance, Dr. Webster will be allocated to work on atmospherics, and Dr. Builder to fading and propagation problems.

2. Work on Fading and the Ionosphere.

The completion of the experimental transmitting equipment at the University of Sydney at the end of last year has made possible a considerable extension of the Board's investigations of fading and the ionosphere.

In the previous work of the Board carried out at Jervis Bay*, it was evident that under the particular conditions that applied, the chief reflecting medium concerned in the return of indirect rays to the earth was a layer at a height of about 110 kms., i.e., the Kennelly-Heaviside Layer. However, definite indications of the effect of the Appleton Layer at a height of about 250 kms. were observed, and it appeared desirable to make further investigations of this reflecting region. For this purpose, a different set of experimental conditions was required.

The opportunity for these observations occurred with the completion of the Sydney University transmitter. Field intensity measurements† of a "B" class station located in Sydney and working at a frequency

* See Radio Research Board—Report No. 2, Council for Scientific and Industrial Research, Bulletin 59.

† Described by Green and Wood in *J. Inst. Eng. Aust.*, Vol. 5, No. 1, January, 1933.

of 1,125 kilocycles per second were used in the selection of a suitable set of conditions for the new Appleton "frequency-change" experiments. As a result of this short survey of both ground and sky wave intensities, it was concluded (i) that the University transmitter should operate at a mean frequency of about 1,500 kilocycles per second, (ii) that a special transmitting aerial should be available, in addition to the normal type, in order to increase the sky wave radiation, i.e., an aerial whose radiator was a horizontal wire one half wavelength long, and (iii) that the existing receiving site at the Military Camp, Liverpool, N. S. Wales, distant 25 kms. from the sender, was suitable.

Preliminary tests at Liverpool showed that, even at this short distance from the transmitter, sky wave intensities were adequate for the "frequency-change" experiments, and during the latter part of 1932 an extensive programme of ionosphere height measurements was undertaken. Observations were made during the greater part of the night, both in Melbourne, where the received signal was due entirely to sky waves, and also at Liverpool, where there was also a ground wave present for reference purposes.

The results obtained at the shorter distance showed that the Appleton Layer was more often the reflecting agent than the Kennelly-Heaviside region; thus it was possible to make a much more extensive investigation of the upper layer than had been possible at Jervis Bay, where penetration of the lower layer was only infrequently observed.

The simultaneous observations made in Melbourne, distant 800 kilometres from the sender, have thrown considerable light on the mechanism of interstate broadcast reception at distances of this order. On those nights when the check measurements at Liverpool indicated that the Kennelly-Heaviside Layer was in operation, the reception in Melbourne was comparatively steady, and the photographic records of the special "frequency-change" tests showed that the received signal was composed of two sky waves of approximately the same amplitude. On other nights, when the Liverpool measurements showed that the upper layer was the chief reflecting agent for sky waves received at short distances, the long distance reception was irregular, variations in signal occurring very frequently; at these times the "frequency-change" tests showed that either two or three sky waves were being received, one of them having a much longer path than the others and being of less intensity.

Although the records taken during this period have not yet been fully analysed, it is possible to form the general conclusions that:—

(i) In the case of long distance reception, slow fading is caused by interference between, and by changes in amplitude of, two sky waves, each returned to the earth by the Kennelly-Heaviside Layer. Fast fading is ordinarily due to an indirect ray from the Appleton Layer.

(ii) In the case of short distance reception, slow period fading seems always to be due to interference between the ground wave and a sky wave singly-reflected from the Kennelly-Heaviside Layer. Fast fading has been traced, at different times of the night and on various occasions, to (*a*) the reception of a doubly-reflected wave from the Kennelly-Heaviside Layer in addition to the singly-reflected wave from the same layer, to (*b*) the singly-reflected wave from the Appleton Layer at times when the main

sky ray is that from the Kennelly-Heaviside Layer, and to (*c*) the doubly-reflected wave from the Appleton Layer at those times when the main sky ray was also from the same layer. There have also been occasions during the sunset period, when the origin of fast fading has escaped detection.

The same field-intensity set which had been used in obtaining data for the operating conditions of the Sydney University transmitter was later converted for measurements at a frequency of 200 kilocycles per second (1,500 metres wavelength), the stations observed being the Naval Board's transmitters VHD and VHJ at Garden Island (Sydney Harbour), and at Flinders Naval Depot, Western Port, Victoria. With this equipment, measurements of ground wave attenuation, night-time severity of fading, intensity of sky wave, and the intensity of atmospherics, have been made in a number of representative localities in south-eastern Australia, both along the coast-line and inland. In consequence, much information regarding the fading of long waves over different types of country and at different distances from the transmitter has been obtained. The results of this work have recently been published (*J. Inst. Eng. Aust.*, Vol. 5, No. 6, June, 1933).

Steady progress has been made at Liverpool with the design and operation of apparatus to measure simultaneously all of the properties of downcoming waves, including length of atmospheric path, angle of incidence at the ground, amount of lateral deviation from the direction of propagation of the ground wave, relative intensities of the normally and abnormally polarised components of magnetic force, angular phase difference between these components, and the sense of rotation of the total magnetic force.

Preliminary results already obtained tend to indicate that the amount of lateral deviation for an east to west direction of transmission is small, but tests of the apparatus have not yet reached the stage when a definite announcement can be made as to the accuracy of the measurements. Concurrently with this experimental work, it has been found necessary to prosecute a number of theoretical investigations concerned with the reception of more than one downcoming wave in addition to the ground wave. The results show that care will have to be exercised in the selection of suitable conditions for these experiments.

3. Work on Atmospherics.

Equipment.—The use of cathode ray direction-finders for observing the intensities of atmospherics and locating their sources was described in last year's report. This work has been continued in co-operation with the Solar Observatory, Mt. Stromlo, F.C.T., particularly with a view to providing more accurate information on the sources shown on the directional recorder charts.

The recorder—which is located at Mt. Stromlo—previously worked on a wavelength of 30,000 metres, and the aerial system rotated once every quarter of an hour. Owing, however, to the good propagation on this long wavelength, it was found difficult to distinguish on the charts between sources at 500 to 5,000 kms. and those at several thousand kms. The wavelength has therefore been reduced to approximately 10,000 metres where the variation of intensity with distance is greater. It was found also that the speed of rotation was too great to record accurately the sources of cyclone activity to the south. Though

these are not very important as regards radio interference, they are of meteorological significance as indicating barometric depressions, generally over the sea. The time of rotation of the aerial system and recording drum has therefore been altered to half an hour. The instrument is now giving reliable and easily interpretable records of all sources distant not more than 2,000 kms. by day and some 5,000 kms. by night.

A similar recorder which was remodelled for the purpose in the workshop of the Natural Philosophy Department of the University of Melbourne has been installed at the Magnetic Observatory at Watheroo in Western Australia, so that, in future, a much more accurate analysis of the sources in and beyond Northern Australia will be possible.

As mentioned in last year's report, an auxiliary receiver operating a cathode ray tube was used to measure the intensity on 300 metres of atmospherics at the same time as their intensities and directions were observed on 3,000 metres on the cathode ray direction-finder, thus enabling comparison of the intensities on the two wavelengths. Further information was obtained by applying a time-base to this cathode ray tube so as to give an indication of the composition and duration of the atmospherics. Information on the duration of atmospherics was obtained also by photographic registration with an Einthoven galvanometer. A Cambridge Thread Recorder was also found to be useful in recording "noise levels" due to a large number of atmospherics at the same order of intensity.

Results.—The analysis of a year's charts of the recorder was described in last year's report and the general conclusions stated. Since then, a more detailed analysis of the close sources—within 2,000 kms. of Canberra—has been carried out. Only days when cathode ray direction-finder observations were also available were considered, as the latter enabled the locations of the sources to be determined. The region within the range of observation was divided into equal areas, and the sources in each area for the year compared as regards the number, average activity, and average duration. The diurnal and seasonal variations of these factors were also studied.

The main points brought out by the analysis are:—

(i) In general, the annual number of sources increases towards the Equator. The topography also affects the number considerably.

(ii) The activity of sources shows a steady increase with approach to the Equator.

(iii) The average duration of sources increases slightly towards the North, but the most noticeable feature is the marked difference between sea and land sources. The average duration for land sources in the area studied is 6 hours. They occur mainly between noon and 6 p.m. For sea sources, the average is 10 hours, and the times of occurrence are distributed much more uniformly over day and night.

(iv) In the North, the land sources are of the tropical type, and are practically confined to the summer months, while in the South they are of the cyclonic type and occur much more evenly throughout the year.

Since this knowledge is available for a considerable part of the continent, it may be utilized to estimate the amount of interference

with radio reception, providing the intensity of the resulting interference is known.

The intensities measured in most of the past work have been peak intensities on the cathode ray tube. These have been compared with the deflections for continuous-wave transmissions, and it has been found that, on a broadcast receiver on a wavelength of 300 metres, the peak intensity corresponds to that which would have been produced by a transmitter located at the source of atmospherics radiating a carrier wave power of the order of 2 kilowatts. It has been found that the peak intensity of an atmospheric is approximately proportional to the wavelength, so that the corresponding power on wavelengths other than 300 metres regarded as a source of interference will vary as the square of the wavelength.

This knowledge is of value in assessing the probable degree of interference to broadcast listening, but several steps are necessary before the final stage of application can be reached. Since the laws of propagation of radio waves are reasonably well understood, it is possible to estimate the electrical quantities that will influence listening conditions at the receiving end, but the interpretation of these quantities in terms of the listener's experience when listening to a programme requires the introduction of psychological and other factors.

Preliminary observations on this aspect showed that, for several individual listeners when listening to a programme of classical music, the interference from occasional atmospherics did not become appreciable until the peak intensity of the atmspherics was at least equal to the carrier-wave intensity of the station received.

The above information is of greatest value for close sources, where the direct ray will predominate. At night, however, the indirect or sky ray becomes of importance, as the intensity due to it is of the same order for distances between 300 and 1,000 kms. The atmospherics will then be received from over a great area, and may be in such great numbers as to give what approximates to a continuous "noise level" in a receiver of sufficient sensitivity. For satisfactory reception, the received signal intensity must be considerably in excess of this level. Tests carried out, using a Cambridge Thread Recorder, on a number of summer nights when some very active sources were being received mainly by the indirect ray, showed that the interference from this cause would be scarcely audible to a listener receiving a programme of music if the carrier-wave field strength of the station was at least 0.5m.v/m. on a wavelength of approximately 300 metres.

The duration and composition of the individual atmospherics is of importance in determining the degree of interference caused, and some preliminary observations on this aspect have been made by using a time-base on a cathode ray tube, and by photographic registration on an Einthoven galvanometer. These have shown that the average atmospheric from a close source consists of a succession of impulses of varied intensity occurring in rapid succession. The total duration of a single atmospheric varies from less than 0.1 second to 1 second, or even more on occasions. The average duration was of the order of 0.4 seconds for those observed.

Future Work.—Preparations are being made for the transference of part of the equipment to a site in Queensland to carry out a more intensive study of the very active sources which occur there in the summer. The frequency of close sources will also permit of more

ready observations of intensities on shorter wavelengths, and of the characteristics of atmospherics and the resultant interference. It will also provide a longer observing base for simultaneous direction-finding and a location which will be particularly suitable for the sea area to the east.

4. Publications.

The following publications have been issued during the past year as a result of the Board's investigations:—

(*a*) *Publications of the Council for Scientific and Industrial Research.—Bulletin* 68.—"Radio Research Board: Report No. 5, Atmospherics in Australia—I.", by G. H. Munro, M.Sc., A.M.I.E.E., and L. G. H. Huxley, M.A., D.Phil.

(*b*) *Individual Articles.*—(For convenience, summaries of those reports are also included).

1. "A Field-Intensity Set," *J. Inst. Eng. Aust.*, Vol. 5, No. 1, January, 1933, by A. L. Green, M.Sc., and H. B. Wood, B.E., B.Sc.

The paper describes a field-intensity set for studying both ground and sky waves. A novel feature, making for simplicity of calibration, was the use of a vacuum-tube millivoltmeter, specially designed to have a high sensitivity. Its use enabled the components of the artificial signal injection apparatus to be calibrated directly; the accuracy of the complete assembly therefore finally depended only on the precise measurement of the geometrical dimensions of the loop aerial, and on the calibration of the vacuum-tube millivoltmeter. The use of a substantially linear vacuum-tube voltmeter, following the amplifier in the receiver, greatly facilitated the measurement of the low field-intensities. A few results of practical tests with the apparatus in measuring field-intensities of both ground and sky waves from a "B" class transmitter located in Sydney are given.

2. "Measurements of Attenuation, Fading and Interference in South-Eastern Australia, at 200 kilocycles per second," *J. Inst. Eng. Aust.*, Vol. 5, No. 6, June, 1933, by G. H. Munro, M.Sc., A.M.I.E.E., and A. L. Green, M.Sc.

The same field-intensity set described above, but converted for use at a wavelength of 1,500 metres, was employed.

(i) *Attenuation.*—Equivalent ground conductivities, in electromagnetic units, were deduced for the following types of country:—For transmission along the coast, ground wave path mainly over sea, the conductivity was 10^{-13} to 5×10^{-13}. Transmission over flat country, conductivity 10^{-13}; over flat wooded country, conductivity 5×10^{-14}. Transmission over undulating wooded country, conductivity 10^{-14}. Transmission over country, including mountain slopes, conductivity 3×10^{-15}.

(ii) *Fading.*—The observations made in the areas where the carrier wave was exhibiting the variations characteristic of fading permit the following deductions to be made for a frequency of 200 kilocycles per second:—For coastal districts, where much of the transmission path is over sea water, the 50 per cent. fading ring is not closer than 550 kms. to the transmitter. For inland districts, where the intervening country is undulating and wooded, 50 per cent. fading is first encountered at about 250 to 350 kms. from the transmitter. For inland districts, where

some part of the transmission path is over mountain slopes, the 50 per cent. fading ring occurs at about 200 to 250 kms. from the transmitter. The greatest values of indirect ray intensity recorded were a little higher than 0.1 millivolts per metre for 1 kw. radiated.

(iii) *Interference.*—The general noise-level due chiefly to atmospherics propagated at night from great distances, did not exceed 0.015 millivolts per metre.

3. "The Limiting Polarisation of Downcoming Radio Waves Travelling Obliquely to the Earth's Magnetic Field," by W. G. Baker, B.E., D.Sc., and A. L. Green, M.Sc., accepted for publication in the *Proc. Inst. Radio Eng., New York,* 5th April, 1933.

The basis of this paper was Radio Research Board, Report No. 3 (Council for Scientific and Industrial Research, Bulletin 60). Since this report was published, however, the theoretical investigation has been extended, and the following summarises the complete paper:—

It is found that, as a downcoming wave leaves the ionosphere, the polarisation tends to a definite limit. The shape of the polarisation ellipse is determined solely by the frequency of the wave relative to the critical frequency and by the angle between the direction of propagation and the lines of force of the earth's magnetic field. The orientation of the ellipse is such that the major and minor axes are perpendicular to the direction of propagation; the major axis is in the plane containing the direction of the earth's field and the direction of propagation. The sense of rotation of the electric vector contained by the ellipse is left-handed when the direction of propagation of the "ordinary" downcoming ray makes an acute angle with the earth's field; the rotation is right-handed when the angle is obtuse. The sense of rotation of the "extraordinary" ray is the reverse. By a simple transformation of the principal components of electric force, namely, those directed along the axes of the ellipse, to components respectively in the vertical plane containing the sending and the receiving stations, and parallel to the ground, it has been shown to be possible to predict the polarisation of a downcoming wave, as measured at the ground, at any given distance and in any direction from the transmitter. As a practical example, maps have been drawn on which there have been marked lines of equi-polarisation, the conditions being those for transmission from station 2BL, Sydney, New South Wales, frequency 855 kilocycles per second. An important theoretical point which appeared in the course of the analysis was that, in the ionosphere, the true direction of propagation is oblique to the wave-front. Practically, this means that a wave propagated obliquely to the earth's magnetic field will be laterally deviated; the amount of deflection depends on the gradient of ionization in the layer.

1. "The Polarisation of Sky Waves in the Southern Hemisphere," by A. L. Green, M.Sc., transmitted to the Institute of Radio Engineers, New York, for publication in the Proceedings.

The article is based on a part of Radio Research Board Report No. 2, Bulletin No. 59 of the Council for Scientific and Industrial Research. It describes the experimental measurement of right-handed polarisation of downcoming waves as compared with Appleton's observations of left-handed rotation in the Northern Hemisphere.

5. Acknowledgments.

Once again, acknowledgment is due to a number of organizations and individuals for the valuable co-operation they have furnished. The help of the Postmaster-General's Department and the Universities of Melbourne and Sydney has been continued on the previous lines. The Department of Defence has afforded valuable assistance in several ways, but notably by the loan of apparatus and the accommodation of equipment at Laverton (Victoria) and Liverpool (New South Wales). The Commonwealth Solar Observatory at Mt. Stromlo and the Watheroo Magnetic Observatory of the Carnegie Institution are also co-operating most helpfully in connexion with the work on atmospherics.

The Composition of Different Regions of Mounds of *Eutermes exitiosus* Hill.

By F. G. Holdaway, M.Sc., Ph.D.

Erratum.

The author of this article, which appeared in the previous issue, has now noted that in portion of the discussion on the lignin : cellulose ratio the ratio was given incorrectly. At the bottom of page 164, the last paragraph should read:—

> "The ratio of lignin to cellulose in its food would therefore be about 1:2, and this ratio has been changed by digestion of cellulose to 4:1. (A similar change from a lignin:cellulose ratio in camphor wood of about 1:2½ to a ratio of about 4:1 in the nest is recorded by Oshima (1919) for *Coptotermes formosanus*. . . ."

Botulism of Sheep in Western Australia and its Association with Sarcophagia.

By H. W. Bennetts, D.V.Sc.

Erratum.

In the article entitled "Botulism of Sheep in Western Australia, and its Association with Sarcophagia," and appearing in the previous issue, it was stated that on one property where green lucerne, rabbit carcasses, and mounds of rabbit droppings were all exposed to a mob of sheep, they showed a decided preference for the droppings. This statement was based on a report from a source which was believed to be quite authentic, and was accepted in good faith. Recently, it has been established that the facts were misstated. No lucerne was exhibited. It has been frequently noted, however, as in this instance, that sheep show a marked appetite for rabbit droppings, which are preferred when only dry feed is available.

PLATE 1.

(*Blue Stain in Pinus radiata* (*insignis*) *Timber.* *See page* 244.)

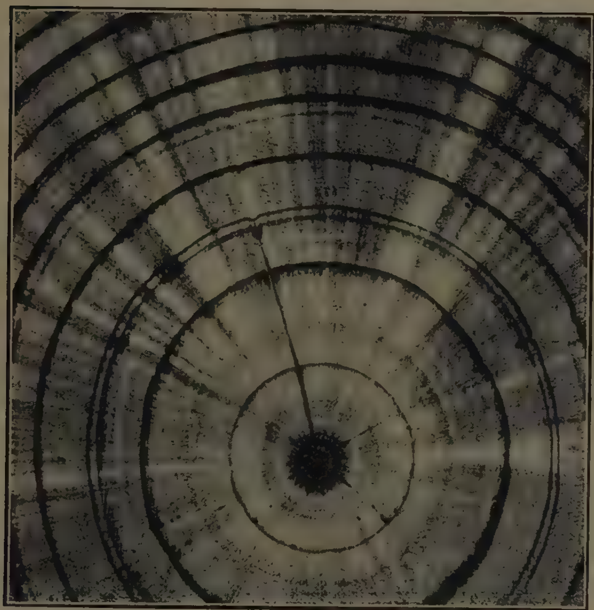

FIG. 1.—End section of a 3″ × 3″ *P. radiata* square showing the penetration of blue stain (the dark radial streaks).

PLATE 2.

(*Downy Mildew (Blue Mould) of Tobacco.* *See page* 260.)

(*Left*).—*N. glauca* Graham. Tree tobacco. A shrub 10 to 15 feet high. Leaves ovate, varying from 1½ to 9 inches in length, fleshy and glaucous with a long slender stalk. Flowers terminal, and in a loose panicle, tubular, greenish yellow, 1½ inches long. Capsule ovoid and similar in appearance to a tobacco capsule. The photograph shows a twig in flower.

(*Right.*)—*N. glauca* attacked by downy mildew of tobacco. The growing shoot has been killed, and new shoots arising from the axils of the leaves have been attacked. The ragged appearance of the large leaf is due to the diseased areas having fallen away. The general appearance is very much like that of tobacco attacked in the autumn.

PLATE 3.

(*Foot and Root Rots of Wheat in Australia. See page* 269.)

Pot Experiment Out-of-doors—Federation Wheat in Sterilized Soil.

(*a*) Plants from grain inoculated with conidia of *Fusarium culmorum*. All seedlings are apparently healthy.

(*b*) Plants from grain inoculated with a mixture of spores of *Fusarium culmorum* and *Urocystis tritici*. Only 45 plants out of 81 standing are apparently healthy, the others, 36, are dying or dead.

(*c*) Plants from grain inoculated with spores of *Urocystis tritici* only. All seedlings are apparently healthy. The photograph of the controls was omitted because in general appearance they were not different from either A or C.

PLATE 4.

(*Fruit Bud Studies. II. The Sultana. See page* 285.)

LONGITUDINAL SECTIONS OF SULTANA BUDS. (× 36.)

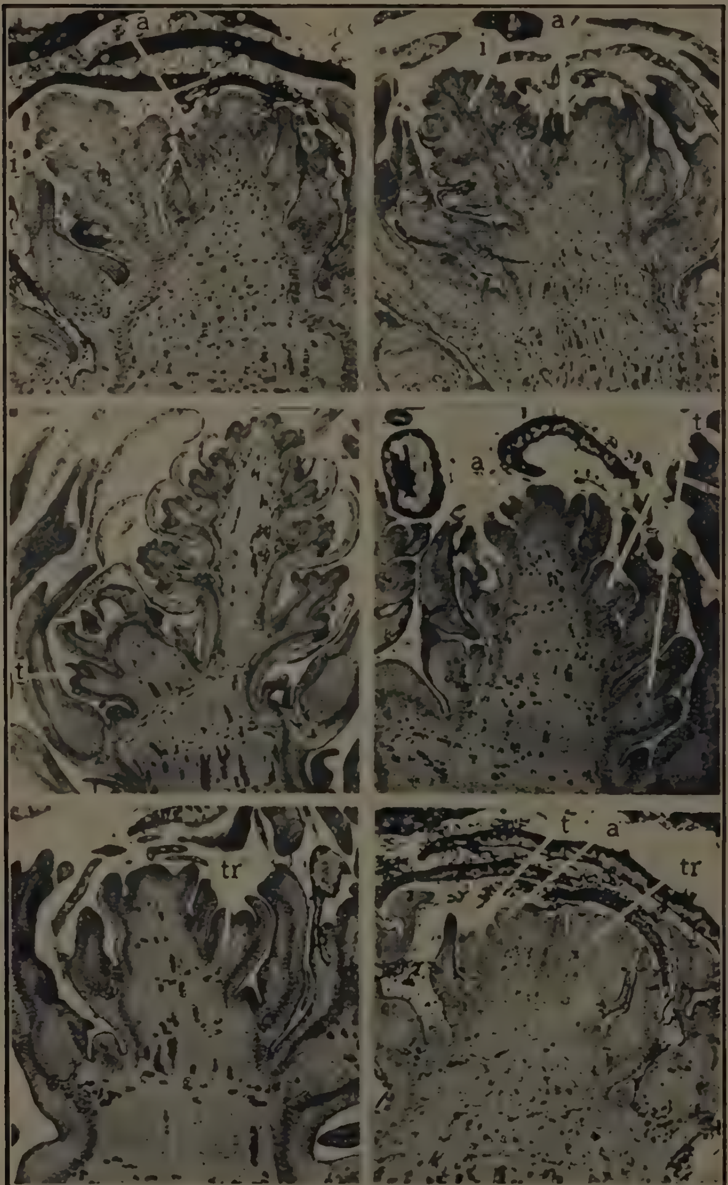

FIG. 1 (top left).—The 7th bud 14.3.30 showing a typical inflorescence primordium (*i*). At "a" is an undifferentiated anlage. FIG. 2 (top right).—Stage reached by most advanced inflorescence primordia by end of August. An anlage at "a". FIG. 3 (centre left).—The inflorescence during rapid growth just prior to opening of the bud. Tendril primordium at "t". FIG. 4 (centre right).—Typical tendril primordia at "t" with an anlage at "a". A bud with no inflorescence, 2nd September. FIG. 5 (bottom left).—At "tr" there is a transition form primordium. FIG. 6 (bottom right).—At "tr" a transition form primordium is shown. At "a" is an anlage, whilst at at "t" an anlage which is differentiating as a tendril primordium is shown.

PLATE 5.

(*Fruit Bud Studies. II. The Sultana. See page* 285.)

See page 285.

SECTIONS OF THE INFLORESCENCE OF THE SULTANA. (× 72, EXCEPT FIG. 6.)

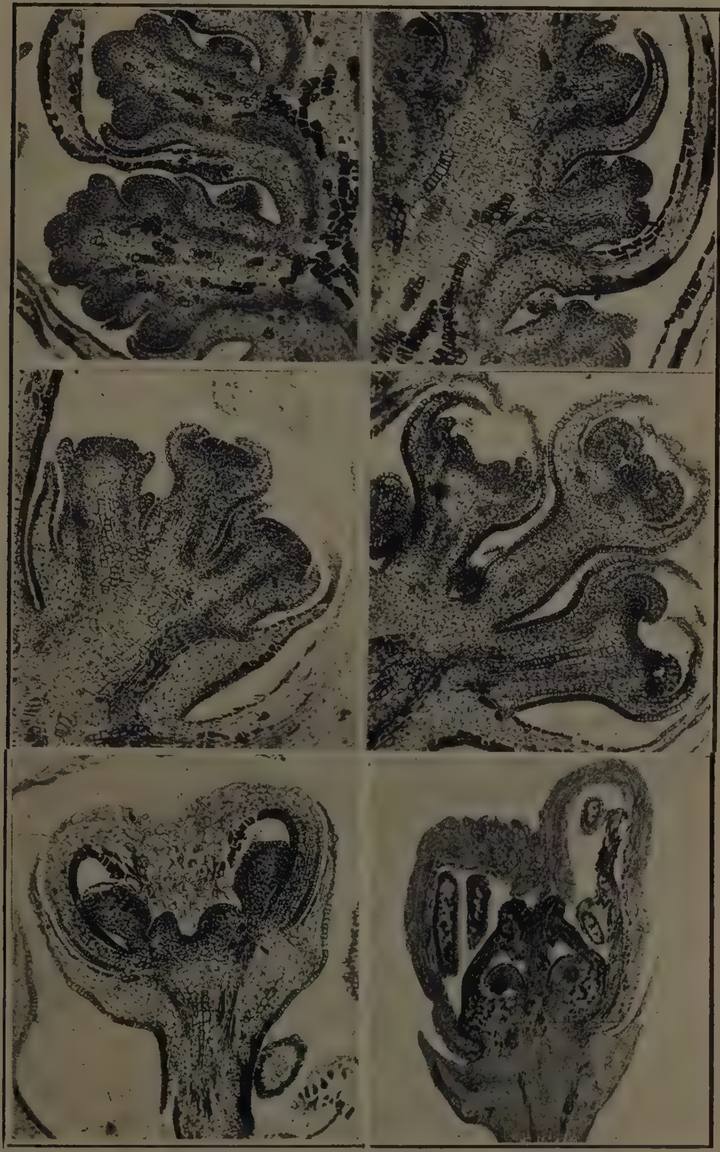

FIG. 1 (top left).—A small portion of the inflorescence represented in Fig. 3, Plate 4, prior to flower formation. FIG. 2 (top right).—The first indication of flower initiation is seen in the division of each growing apex into three. FIG. 3 (centre left).—Three flower primordia with sepals just formed. 8th September, 1930. FIG. 4 (centre right).—The sepals, petals and stamens formed in three flowers. The petals curving inwards prior to fusing. 23rd September, 1929. FIG. 5 (bottom left).—A single flower showing origin of the carpels and petals fused 2nd October, 1929. FIG. 6 (bottom right).—The flower on the 13th October about a fortnight before bloom. Mature pollen is present in the anther. (× 36).

PLATE 6.

(The Microphotography of the Living Pleuro-Pneumonia Virus of Cattle. See page 299.)

Pleuro-Pneumonia "virus" taken by dark ground illumination.

Fig. 1.—Long mycelium with tuft of branches at each end.—Total length about 45μ. Cult. in V.F.-O.S., 20 hours old. × 1250.

Pleuro-Pneumonia "virus" taken by dark ground illumination.

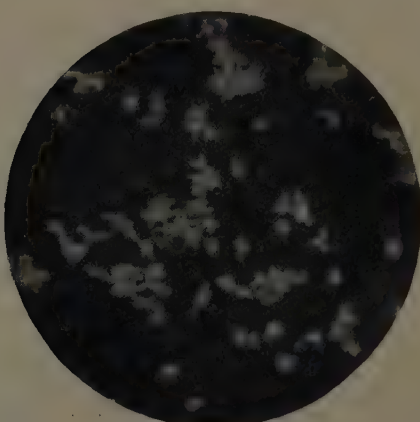

Fig. 2.—Cult. in V.F.-O.S., 20 hours old, showing mycelial fragments and spherical form. × 1250.

NOTES.

The Register of Agricultural Research.

One of the first recommendations of the Standing Committee on Agriculture (see this Journal **1**: 58, 1927), which is representative of the various State Departments of Agriculture and of the Council, was that a record of the individual agricultural researches in progress in various localities in Australia should be prepared and kept up to date from time to time. It was considered that this record would be useful in connexion with the general movement for the dissemination of the results of scientific research and for the avoidance of overlapping.

Some years ago, the first record was prepared under the title "The Register of Agricultural Research." The various State Departments of Agriculture, Universities, the Council, &c., have recently brought the relevant information concerning their research work up to date, and a new Register has been prepared. Copies have just been distributed to State Departments of Agriculture, Universities, the Council, and main Public Libraries throughout the Commonwealth. In addition, a few copies have been sent to Great Britain for the information of agricultural research authorities there.

It will be quite impossible to supply any particular investigator with a copy, as the Register has not been printed. Copies are available for perusal, however, on application to the organizations mentioned in the preceding paragraph.

The Division of Forest Products—Visitors to Laboratories.

Early in November, Mr. A. T. J. Bianchi, Officer-in-charge, Technology Section of the Forest Research Institute, Buitenzorg, Java, will commence a stay of some months at the laboratories of the Division of Forest Products. This Institute proposes to erect experimental seasoning kilns and commence a series of investigations into the kiln seasoning of the timbers of the Dutch East Indies. While at the laboratories of the Division, therefore, Mr. Bianchi will make a comprehensive study of the Division's seasoning research, as well as of the types of kilns in commercial use in Australia.

Accompanying Mr. Bianchi will be one of the engineers of the Royal Packet Navigation Company, Mr. C. J. Andriesse, who will take an active part in the erection and operation of a trial kiln which is to be erected at a commercial plant in addition to one or two laboratory kilns at the Research Institute.

Mr. F. Gregson, Utilization Officer of the Western Australian Forests Department, is also spending two months in the Division to study the methods used in attacking problems of utilization and seasoning.

The Gas Storage of Foods: A Review of the Present Position.

A paper on the above subject by Dr. Franklin Kidd, M.A., D.Sc., of the Low Temperature Research Station, Cambridge, was read on the 14th February last before the North-West Branch of the British Association of Refrigeration, and has been published in the *Proceedings of the British Association of Refrigeration,* Vol. XXIX., No. 2, 1932-33, pp. 130-145.

The first investigations by the British Food Investigation Board on gas storage were undertaken as a result of a series of publications in 1914 on the influence of carbon dioxide upon the germination, respiration, and life duration of seeds. At the outset, the dominating idea was that atmosphere control might prove effective without temperature control, and thus serve as an alternative method to the use of cold storage. This idea was soon shown to be without foundation. It was also realized that different products would differ in their reaction to atmosphere control, and it was decided to confine investigations in the first place to one kind of fruit, namely, the apple. Every year, only one set of apple storage experiments can be carried out, and hence progress was slow.

In the course of two or three years, the experiments were developed to the scale of a chamber holding 8 tons, but without temperature control. At this stage of the work, several facts emerged. The first was that under the conditions of restricted ventilation with fresh air many varieties tended to scald badly in gas storage, though otherwise well preserved.

Scald is a superficial browning of the skin, which greatly disfigures apples and renders them susceptible to fungal rotting. Another important fact brought to light by these experiments was that in bulk storage without temperature control the self-heating of the fruit was a serious factor. During cold weather, the average inside temperature in the store was about 50° F. higher than the outside temperature, whilst during warm weather the difference was over 10° F. It was found that the beneficial effect of the atmosphere control in retarding the ripening was counteracted by warm autumn temperatures in the early days of storage. An experiment was accordingly made in which refrigeration was used only in the hot weeks of early autumn. The results of these trials were unexpected. Some of the varieties of apples used were found to be seriously affected by a new type of injury like that known as low temperature internal breakdown. In contrast with this, the other varieties were preserved in a manner and to a degree far exceeding that possible with cold storage alone.

This experiment definitely marked the end of the idea that gas storage without temperature control was an alternative method to ordinary cold storage. It cannot be too definitely stated that, for nearly all purposes, temperature control is essential in the gas storage of fruit.

By this time, the Low Temperature Research Station at Cambridge had been completed, and it was thus possible to make use of greatly improved facilities for more accurate and comprehensive experiments. The variety of apple used was the Bramley's Seedling, which is the most important English culinary variety. The experiments showed very clearly that, for Bramley's Seedling, 10 per cent. oxygen and 10 per cent. carbon dioxide at 40° F. gave results much superior to those of cold storage and air. As soon as the results of these experiments, which were carried out in 1926-27, became known, growers began to embark

upon the construction of refrigerated gas stores for the storage of Bramley's Seedling. There are now five large commercial gas stores in operation in Kent.

Though the economic possibilities of gas storage for fruit were thus definitely established, a great deal of further work had nevertheless to be done. With this in view, the Ditton Laboratory of the Food Investigation Board has been equipped for semi-large scale gas storage work. In two years, a great deal of further information has been obtained. One of the most instructive and useful lessons has been the demonstration of the great ease with which gas injury may be incurred if safety limits either of oxygen or carbon are overstepped. Moreover, the critical importance of temperature in regard to gas tolerance limits has been strongly emphasized. It has been a general rule to find serious gas injury at 34° F. (breakdown type) and at 50° F. (brownheart type) and none at all at 39° F.

Turning to recent developments with regard to the gas storage of meat and fish, Dr. Kidd states that it has been fully demonstrated that the time limits for the chilled storage of these foods can be considerably extended by the use of atmospheric control in addition to low temperature. The reason for this is quite simple. The time limits of cold storage are set in the main by the activity of living micro-organisms —fungi and bacteria. It is these living organisms which cause putrefaction, taint, and rancidity. If the vital activity of these organisms can be slowed down or stopped by controlling the carbon dioxide and oxygen content of the atmosphere, a very promising line of development is opened up.

As regards pork, chilled storage life can be doubled by the use of high percentages of carbon dioxide. Pork has been kept in perfect condition for over two months at 0° C. in commercial carbon dioxide, and thereafter tasted better than fresh pork, as it was more tender.

At the Torry Research Station, Aberdeen, it has been found that fish packed in ice and stored in air was definitely sour and stale after fourteen days, whereas similar fish packed in ice and stored in carbon dioxide showed no signs of sourness after 28 days.

Beef, when stored in air at 29.5° F., was spoilt in 30 days, but when stored in an atmosphere of 10 per cent. carbon dioxide was in good condition at the end of 60 days. These results have been followed up on a semi-commercial scale by Dr. J. R. Vickery with very successful results. (See article on page 233.)

Similar successful results have been obtained in the storage of unsmoked mild cured bacon, which has been held in perfect condition for eighteen weeks at 32° F. Gas storage of eggs is an established commercial procedure. In Europe, there are a number of stores using this process.

Carbon dioxide and oxygen are among the most obvious cases of control in the storage atmosphere. There are, however, many gases and vapours which are likely to have physiological effects upon fresh foods in storage, such, for example, as ethylene, ozone, ammonia, aldehydes, and alcohol vapours. Until recently, experiments with such substances were conducted in a haphazard manner without much attention to the critical factor of the exact dosage required to produce specific effects. To-day, progress is being made because attention is being directed to the strength and duration of treatments.

Changes in Kiln-Dried Timber during Transit from Australia to England.

Contributed by W. L. Greenhill, B.E., Division of Forest Products.

During recent years, the quantity of seasoned timber shipped from Australia to England has been steadily increasing. The conveyance of seasoned timber by boat immediately raises the question of the probable change in moisture content of the timber which may occur during transit. It has been commonly thought that an increase will occur, and buyers and sellers, therefore, have frequently inquired as to the most suitable moisture content for shipment, so as to provide for delivery of the timber as specified. For this reason, an investigation of the changes which are normally to be expected in kiln-dried timber *en route* has been carried out by the Division of Forest Products with the co-operation of the Forest Products Research Laboratory, Princes Risborough, England, and two of the principal shipping companies concerned.

The procedure in the investigation has been to prepare sample boards from a consignment of timber which is to be shipped to England. The sample boards are weighed and measured, and then distributed in bundles amongst the timber as it is loaded into the boat. Upon arrival in England, the samples are collected, again weighed and measured, and then tested for moisture content. Data relating to the conditions in the hold during the voyage, to other cargo in close proximity, and to the general state of the shipment on unloading are also collected.

Two test shipments of the above nature have now been completed. Both consisted of kiln-dried and re-conditioned machined Victorian mountain ash flooring, which was handled in bundles of 37 boards each. Three sample bundles, containing the same number of boards, but only 3 feet long, were distributed in each consignment. The sample bundles were carefully end-coated to ensure that any moisture changes in these short lengths would be comparable with the changes in the rest of the shipment, in which the lengths ranged from 9 to 20 feet. In both shipments, the stowage conditions were considered very satisfactory, good ventilation being provided throughout the voyages. In the first shipment, the timber was in an insulated hold with a quantity of wool; in the second, it was stowed with a quantity of other timber. The two voyages occupied 55 and 38 days respectively. A summary of the moisture content changes and changes in the widths of the sample boards during the two voyages is given in the following table:—

Shipment.	Bundle.	Average moisture content of boards in bundle. When Shipped.	Average moisture content of boards in bundle. On Arrival.	Average change in moisture content.	Average width of boards in bundle. When Shipped.	Average width of boards in bundle. On Arrival.	Average change in width.
		%	%	%	inches	inches	inches.
1	1	14·3	14·1	−0·2	3·26	3·25	−0·01
	2	12·7	12·7	0	3·26	3·26	0
	3	12·4	12·6	+0·2	3·27	3·26	−0·01
2	1	13·6	13·3	−0·3	3·26	3·26	0
	2	13·1	13·1	0	3·26	3·26	0
	3	13·2	13·2	0	3·27	3·27	0

It will be seen that during transit the moisture changes of the bundles as a whole were negligible. The indicated changes in widths of boards are within the limits of accuracy imposed by the methods of measuring. Both tests serve to indicate that, if properly stowed, timber shipped from Australia at moisture contents from 12 to 14 per cent. is unlikely to change to any appreciable extent during transit to England.

Tests of a similar nature have been carried out by the Forest Products Laboratories of Canada, Vancouver Laboratory, on changes in kiln-dried timber during shipment from Canada to various parts of the world, including Australia, and similar results have been obtained.

Recent Publications of the Council.

Since the last issue of this *Journal,* the following Bulletins and Pamphlets of the Council have been published:—

Bulletin No. 74.—" Observations on Soil Moisture and Water Tables in an Irrigated Soil at Griffith, N.S.W.," by Eric S. West, B.Sc., M.S.

The investigations discussed form part of a programme of work which the Council is carrying out at Griffith in co-operation with the Water Conservation and Irrigation Commission of New South Wales. After the structure of the soil and such factors as the field capacity, the " sticky point," and the wilting point have been dealt with, the effect of weather conditions on the water table is considered at length. A subsequent section deals with the effect growing plants have on soil moisture and the water table. The value of lucerne as a means of controlling a rising water table, and thus of obviating harmful effects on citrus, is shown.

Bulletin No. 76.—" A Soil Survey of the Hundreds of Laffer and Willalooka, South Australia. Report of the Division of Soils," edited by J. K. Taylor, B.A., M.Sc.

The publication deals with the survey of an area in South Australia representative of some 6,500 square miles of country in South Australia and some 4,300 square miles in Victoria, all under a 15 to 20 inch rainfall. Three soil types have been named and described in full. It is shown that, with the exception of a small proportion of the area, the bulk of the soils offer very small prospects of economic returns at the present time.

Pamphlet No. 43.—" Investigations on the Buffalo Fly, *Lyperosia exigua* de Meij." I. " The Host Preference of *L. exigua* (resumé)," by Dr. B. J. Krijgsman and G. L. Windred, B.Sc.Agr.; II. " The Relation between the Adult *L. exigua* and Mammalian Faeces," by Dr. B. J. Krijgsman and G. L. Windred, B.Sc.Agr.; III. " Some Food Reactions of the Larvae of *L. exigua,*" by G. L. Windred, B.Sc.Agr.; and IV. " The Influence of Moisture on the Larvae of *L. exigua,*" by G. L. Windred, B.Sc.Agr.

The Pamphlet gives the results of some of the work carried out at the State Veterinary Institute, Buitenzorg, Netherlands East Indies, on the life history and habits of the buffalo fly. The investigations were carried out by officers of the Council, who were kindly accommodated at the Institute, as part of the Council's general inquiry into the Australian buffalo fly problem.

Pamphlet No. 44.—"The Chemistry of Australian Timbers: Part 3.—The Chemical Composition of Four Pale-coloured Woods of the Genus Eucalyptus—*E. gigantea, E. obliqua, E. regnans,* and *E. sieberiana*" (Division of Forest Products—Technical Paper No. 9), by W. E. Cohen, B.Sc., A. G. Charles, and A. B. Jamieson, M.Sc.

The Pamphlet covers a continuation of the analytical work the Division of Forest Products is carrying out on the Australian eucalypt timbers. It is of particular interest in that it deals with the group of species which have proved to be most suitable for paper making in Australia. Some regular differences in certain chemical factors, such as total pentosans, percentages of "solubles" in various solvents, and "soluble" ratios, were found, and the possibility of employing these as an aid to identification is indicated. In addition, variation of chemical composition within a species was studied, and its possible influence on wood pulp yields is discussed.

Pamphlet No. 45.—"Australian Export Apple Cases" (Division of Forest Products—Technical Paper No. 10), by W. M. Carne and R. F. Turnbull, B.E.

This publication gives the results of an extensive series of tests carried out largely at the instance of the Standards Association of Australia and aimed at the ultimate standardization of the Australian export apple case. The two main types of case now in use, namely, the Canadian and the Australian dump, are described, and the disadvantages of using more than one type are stressed. Data regarding the protective value of the different types (based on the damage occurring during packing and in drop tests), the strength of cases, and the effect of cool storage conditions on case timbers, are presented. It is shown that under the conditions of packing used the dump shape gives better protection to its contents than the Canadian shape, and that unduly tight packing is found to be a source of bruising. Bruising can be reduced by the use of all-round corrugated strawboards. It is recommended that a standardized dump case not less than 18″ x 9″ x 14¼″ in internal measurements be adopted as the standard export apple case.

Forthcoming Publications of the Council.

At the present time, the following future publications of the Council are in the press:—

Bulletin No. .—"Studies in the Phosphorus Requirements of Sheep—I.," by C. J. Martin, M.D., D.Sc., F.R.S., and A. W. Peirce, B.Sc.

Bulletin No. .—"Methods for the Identification of the Pale or Light-coloured Woods of the Genus *Eucalyptus*" (Division of Forest Products—Technical Paper No. 12), by H. E. Dadswell, M.Sc., Maisie Burnell, B.Sc., and Audrey M. Eckersley, M.Sc.

Pamphlet No. 46.—"The Holding Power of Special Nails" (Division of Forest Products—Technical Paper No. 11), by Ian Langlands, B.E.E.

Pamphlet No. .—"Properties of Australian Timbers—Part I." (Division of Forest Products—Technical Paper No. 13). Collated and edited by H. E. Dadswell, M.Sc.